# Arthropod Predation in *Brassica* Agroecosystems: Effects of Latitude, Community Composition, and Diet Breadth

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**Dedication**

To my grandmother, Gladys Louise Rowley

**Abstract**

Arthropod predators provide crucial pest management services by consuming herbivore prey in agroecosystems. Yet, variation in arthropod predation strength among cropping systems and regions can prevent farmers from taking advantage of this alternative pest management strategy. This research examines underexplored potential causes of variation in arthropod predation in agroecosystems. Arthropod predation increases at lower latitudes. However, it is unknown whether this gradient extends to agroecosystems. Diet breadth of an arthropod predator can influence whether a predator can adequately control resident herbivore populations and can impact predation in a community context by determining whether predatory taxa will compete over shared prey, attack each other as intraguild prey, or partition herbivore taxa into distinct prey niches.

To assess the effect of latitude, I first compared predation rates on live and artificial sentinel prey in *Brassica* agroecosystems between the tropical Federal District, Brazil and temperate Minnesota, United States. Contrary to expectations, I found that predation rates on all bait types were similar between the two localities and that reduced predation rates in the Federal District may be related to higher prey densities. Next, to further explore latitudinal effects, I assessed predation rates in *Brassica* agroecosystems across 15 degrees of latitude in the United States and 21 degrees of latitude in Brazil. Surprisingly, my results revealed a reverse predation gradient whereby arthropod predation increased with latitude in both countries. To examine the role of diet breadth, I first tested methods to improve molecular gut content analysis of arthropod predators by verifying broad metabarcoding results with species-specific melting curve analysis. This study documented common false positive and false negative taxonomic results and suggested that a species-specific verification step is necessary to ensure accurate depictions of arthropod trophic interactions. Lastly, I use the results of the gut content analysis to characterize the diet breadth of three coccinellid predator species (*Coleomegilla maculata*, *Hippodamia convergens*, and *Harmonia axyridis*) collected from a *Brassica* agroecosystem in Minnesota. I found that both herbivore and intraguild prey consumption were common among coccinellid species, but that *C. maculata* was least likely to engage in intraguild predation of fellow coccinellids.

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## Introduction

Farmers have always faced the risk of crop loss to insect herbivore pests, however shifts in temperature and precipitation regimes from climate change will likely intensify such pest threats (Deutsch et al. 2018). Damage from insect pests currently reduces crop quality and quantity by 18-26% and costs farmers an estimated $470 billion in lost revenue (Oerke 2006, Culliney 2014). These losses could be exacerbated by warming trends which favor insect reproduction and range expansion, and increasing insect pest resistance to chemical controls (Jamieson et al. 2012, Sparks and Nauen 2015). Alternative pest management strategies, such as employing arthropod predators and parasitoids to regulate herbivore prey populations, could play an important role in creating resilient agroecosystems. Yet, wider farmer adoption of arthropod predation as a pest management method is inhibited by inadequate ecological information in some regions and continued unexplained variability in predator function in others (Riley et al. 1998, Ziska 2014). Persistent questions of where farmers can depend on arthropod predators to protect crops and which predators can best ensure control will continue to stymie acceptance of this alternative pest management strategy if left unanswered.

This dissertation is divided into two sections that are aligned in their work to improve understanding of arthropod predator function in agroecosystems. First, I explored whether a documented pattern of stronger arthropod predation at lower latitudes in natural ecosystems persists in a model *Brassica* agroecosystem. In natural ecosystems, stronger arthropod predation is thought to contribute to species diversity by preventing any one prey species from becoming dominant and protecting plants from excessive herbivory (Pianka 1966, Paine 1966). Despite the importance of arthropod predators in protecting crop plants globally (Shennan 2008), it is unknown whether latitude contributes to predation strength variability in these human modified systems. In the second section of my dissertation I examine metabarcoding as a molecular gut content analysis method for arthropod predators and explore how food webs built from molecularly validated prey consumption could improve knowledge of which predator assemblages are most capable of suppressing prey. Metabarcoding has been increasingly employed in conservation biology to detect the presence of bioindicator freshwater

invertebrates (Elbrecht and Leese 2017a) and to dissect vertebrate diets containing arthropods (Deagle et al. 2006). However, key technical aspects of this technology including primer design, bioinformatic processing, and interpretation of results must be addressed before it can be readily employed in sensitive studies of applied arthropod predation for herbivore pest management.

My dissertation presents fundamental knowledge on the altered relationship between latitude and arthropod predation strength in agroecosystems compared to natural ecosystems (Chapters 1 and 2), describes a novel combination of methods to improve the reliability of metabarcoding for gut content analyses of arthropod predators (Chapter 3), and explores how differential prey use among species within an important agricultural predator family may support the capacity of predators to suppress herbivore prey (Chapter 4).

**Latitudinal gradients in biotic interactions**

Geographic variation in the strength of interactions among organisms has long been cited as a potential cause of the famous latitudinal gradient in species diversity. Naturalist Alfred Russell Wallace first described biotic interaction gradients in the 19$^{th}$ century, and by the mid-20$^{th}$ century, biologists theorized that the strength of biotic interactions at differing latitudes was related to the relative stability or instability of abiotic factors in an environment (Dobzhansky 1950, Fischer 1960). Numerous studies have attempted to validate whether latitudinal gradients in competition, mutualisms, herbivory, and predation exist in natural ecosystems, with mixed results (Schemske et al. 2009, Moles and Ollerton 2016). Variation in results by ecosystem, interaction type, and focal study species limits the ability to discern if biogeographic patterns exist and to understand what factors may give rise to such patterns. Global scientific networks are increasingly overcoming this challenge by creating widely distributed standardized experiments (Borer et al. 2014, Roslin et al. 2017). Still, most studies examine latitudinal biotic interaction gradients to better understand natural ecosystems and leave agroecosystems unexamined.

**Ecological function**

To understand how the presence or absence of an organism influences processes within an ecosystem, the function of that organism must be well defined (Cadotte et al. 2011). Functional diversity rather than species diversity has been increasingly employed to understand how species with differing ecological natures complement, make redundant, or antagonize each other within a trophic guild (Duffy 2002, Nunes-Neto et al. 2014). By quantifying functional traits of specific species and measuring the differences and overlaps in traits among species, ecologists are improving predictions of which biological communities can provide ecosystem services such as pollination, decomposition, or pest control (Blüthgen and Klein 2011, Greenop et al. 2018, Francioli et al. 2020). In the applied contexts, understanding how members of a functional group interact under varying biological and climate constraints could reveal management actions to support organisms which contribute most to a desired ecosystem service.

**Diet breadth**

The identity and quantity of taxa consumed by an animal reveals not only its direct interactions, but also its indirect relationship to other animals through shared predators or prey (Jiang and Morin 2005). Diet breadth can also uncover how flexible an animal is in the face of environmental changes, how quickly it responds to altered resource availability, and the habitats it moves through while foraging (Altermatt 2010). Determining diet breadth can be difficult for certain taxa, particularly if digestion obscures food identity or diet samples are difficult to collect (Pompanon et al. 2012, Furlong 2015). Laboratory studies can reveal what an animal may consume, but only field studies can uncover what an animal actually eats (Langellotto and Denno 2004, Roubinet et al. 2017). Determining the precise diet of an animal in the field is particularly important when the consumption of a given taxa may be a desired outcome and consumption of alternative taxa may reduce the likelihood of that desired outcome.

**Arthropod predator function in agroecosystems**

Arthropod predators play a crucial role in regulating pest herbivore prey populations in agroecosystems (Naranjo et al. 2015). Management techniques in agroecosystems often create plant communities dominated by few, densely planted crop

plant species, which are well nourished by irrigation or fertilization practices. On these high-quality, abundant crop plants, arthropod herbivore populations may reach pest status if left unchecked by predators and parasitoids (Altieri and Letourneau 1982). The same conditions that benefit arthropod herbivores in agroecosystems can prevent the presence of arthropod predators (González-Chang et al. 2019). Many arthropod predators require alternative shelter, nectar, prey, or pollen to persist and reproduce in a habitat, all of which can be lacking in simplified agroecosystems (Gurr et al. 2017). However, arthropod predators can vary in response to efforts to enhance the availability of required resources based on functional traits including mobility, habitat domain, and diet breadth (Greenop et al. 2018). For example, predators may not be capable of locating crucial resources if located outside the range of predator movement or may be prevented from utilizing resources if intraguild competition or predation risk are high. Determining the conditions within a given agroecosystem that best support complementarity rather than antagonism among predators is at the core of leveraging predator capacity regulate herbivore populations.

***Latitudinal gradients in arthropod predation***

Evidence from natural ecosystems largely supports a latitudinal gradient in arthropod predation, with stronger predation rates at lower latitudes (Jeanne 1979, Novotny 2006, Roslin et al. 2017). Yet, most of the habitats surveyed in these studies were forested, and almost all of them were in natural ecosystems. These survey constraints overlook the fact that the majority of land coverage globally is under cultivation (FAO 2016). It is in these agroecosystems, where humans may benefit most from understanding underlying patterns in arthropod predation. In the absence of arthropod predation, crop losses to herbivores may put the global food supply at risk (Naranjo et al. 2019). Research has documented that regional landscape diversity can impact arthropod predation rates, but has yet to explore if latitude could contribute to variable arthropod predation in agroecosystems (Thies and Tscharntke 1999, Bianchi et al. 2006). Agroecosystems experience the same gradients in temperature and daylight as natural ecosystems but can deviate significantly in the structure of plant communities that form the foundation for arthropod interactions.

### *Diet breadth and arthropod predation*

The diets of arthropod predators are notoriously difficult to assess in field populations. Their small and cryptic feeding habits can obscure common interactions by making predatory attacks difficult to observe or preventing identification of remnant prey within predator guts (Furlong 2015). Understanding diet breadth is crucial to assessing the functional role that an arthropod predator plays in an agroecosystem, identifying if it consumes herbivore and or intraguild prey (Jiang and Morin 2005, Paula et al. 2016). In the past, arthropod predators have been identified as specialists or generalists, but these categories do not provide sufficient information to predict the impact of a predator (Symondson et al. 2002). Prey consumption by predators can vary by developmental stage, habitat preference, or interactions with other predators. Co-occurrence in the field can imply that a predator may eat a given prey taxa, but it is not sufficient to confirm consumption. In agroecosystems, when the desired outcome of arthropod predation is to regulate herbivore populations, prey identity is consequential. If a predator consumes feeding guild members, the presence of that predator can actually lower the total level of pest control in a habitat (Rosenheim 1998). Historically, identifying these differences has been difficult, but advances in molecular methods now facilitate fine-grained analysis of diet breadths to confirm predator prey interactions.

## Chapters

Chapter 1 and 2 explore if latitudinal patterns in arthropod predation hold in *Brassica* agroecosystems. In Chapter 1, I compared predation rates on live and artificial sentinel prey baits in *Brassica* dominated plots between the tropical Federal District, Brazil and temperate Minnesota, United States. I deployed live aphids, lepidopteran eggs, caterpillars, and artificial caterpillars and found that predation rates were similar between the two localities for each sentinel prey type. In the Federal District, my results suggest a reduction in predation rate from tropical natural ecosystems that may be attributable to high prey densities (Roslin et al. 2017). In Chapter 2 I assessed predation rates on artificial caterpillars in *Brassica* agroecosystems across 36º latitude, spanning 5 localities in the United States and 5 localities in Brazil. Surprisingly, my results show a reverse

predation gradient in agroecosystems, whereby arthropod attacks are stronger at higher latitudes. However, in each country, the rate of increased predation with latitude and the underlying causes of this pattern were distinct, indicating that there may be biogeographic differences in the functional organization of arthropod predation in agroecosystems.

Results from the field studies in Chapter 1 and 2 provide a foundation for further exploration of latitudinal biotic interaction patterns in agriculture and evidence to support examining latitude as a factor contributing to variable effects of arthropod community composition on predation rates. These field studies highlight persistent knowledge gaps that may prevent farmers from relying upon arthropod predators for pest control services and promote continued reliance upon chemical controls. My results also raise important questions about the transferability of agronomic recommendations from one region to another and whether geographic biases in agroecological research could limit understanding of how to support pest management in tropical regions.

Chapter 3 delves into technical aspects of validating the diet of an arthropod predator under field conditions by assessing the efficacy of commonly used metabarcoding methods with an independent species-specific molecular verification step. I designed in-house universal primers aimed at detecting the full array of potential arthropod prey in agroecosystems and evaluated primer accuracy in detecting prey from the guts of coccinellid predators compared to other commonly used metabarcoding primers. My assessment of primers relied upon validating taxa detected by metabarcoding with species-specific melting curve analysis. By probing into the source predator DNA I determined that both false positives and false negatives are persistent and problematic in diet analysis metabarcoding workflows. Furthermore, my results highlighted that read abundances are a poor indicator of whether a taxonomic hit is a true positive detection and encourage independent validation of metabarcoding results to avoid removing valid, but rare taxa and retaining false, but common taxa in ecological results.

This technical work underscores the promise and pitfalls of relying upon molecular tools to better understand trophic relationships in agroecosystems. While species-specific sequencing of predator gut contents has become commonplace in ecological entomology, these studies primarily focus on prey species of economic

concern. Arthropod predators, such as coccinellids may consume several prey types throughout the growing season and in order to predict which predator taxa will reliably control herbivore pests it is vital to detect all types of prey consumption. Increasingly, metabarcoding is being used in biosurveillance programs to detect pests, invasive insects and potential predators (Piper et al. 2019, Sousa et al. 2019, Westfall et al. 2020). My results show that reliability of trophic relationships revealed by such molecular diet analyses of arthropod predators may be improved by combining taxonomically broad and narrow molecular approaches.

Coccinellid beetles were identified as important predators in the *Brassica* agroecosystems studied in both Chapter 1 and 2 of this work. Chapter 4 explores the functional trait of diet breadth in three coccinellid species (*Coleomegilla maculata*, *Harmonia axyridis*, and *Hippodamia convergens*). By analyzing diets of individual coccinellids contributing to pooled samples used in Chapter 3, I determined that consumption of herbivore and intraguild prey depends on coccinellid species and diet breadth. While all coccinellids consumed an array of herbivore prey, only *C. maculata* consumed herbivore prey likely to damage a focal *Brassica* crop. Further, my results show that intraguild predation was common among all three species tested, but that *H. convergens* was most likely to consume other coccinellids. A broader diet proved to be beneficial and detrimental to the predator function of herbivore control. *C. maculata* was more likely to consume *Brassica* herbivore prey, whereas *H. convergens* was more likely to consume intraguild prey with a broader diet. Unexpectedly, my results show non-resident prey consumption by *H. convergens* which suggest recent immigration from the surrounding habitat. This finding raises important questions about the role of arthropod predator movement in predator function.

Results from Chapter 4 call attention to the richly varied diets of arthropod predators. Even within a single subfamily (Coccinellinae) and agroecosystem, species can vary in which prey species they consume. Many studies attempt to integrate aspects of predator functional diversity into assessments of pest control capacity, but to date few have addressed the nuances of diet breadth (Greenop et al. 2018). Moving beyond dichotomous categories of ‘specialist’ and ‘generalist’ will allow researchers to more readily identify the individual or group of predators most likely to reliably provide

herbivore pest control services. Molecular tools such as the metabarcoding and melting curve analysis employed in Chapter 3 and 4 will improve capacity to assess dietary overlaps and expand food webs to include novel and underexplored prey groups.

In summary, my dissertation advances understanding of latitudinal variability in arthropod predator function, develops technical aspects of molecular gut content analysis, and improves knowledge of trophic interactions among coccinellid predators and prey in *Brassica* agroecosystems. Scientific knowledge gained from my work will improve assessments of the capacity of arthropod predators to manage pests in agriculture and will motivate future integration of biogeographic theory and molecular tools into agroecological entomology.

## Chapter 1: Arthropod predation strength is similar between a temperate and tropical *Brassica* agroecosystem.


### Summary

Arthropod predation is stronger at lower tropical latitudes in natural ecosystems, yet it is unknown if this pattern holds in human managed agroecosystems. In both natural and agroecosystems arthropod predators provide crucial pest management services, protecting plants from excessive herbivore damage. Agroecologists recognize that predation strength can vary based on the availability of key predator resources. However, it is unclear if biogeographic context influences predator resource availability in agroecosystems, and how the predator community would respond to such resource variation in differing climates. This study employs predator-exclusion cage studies and sentinel prey to test whether biogeographic patterns in arthropod predation hold in tropical versus temperate agroecosystems dominated by *Brassica oleracea* (L.). Contrary to trends in natural systems, results from this study show similar rates of predation on aphids, lepidopteran eggs, and artificial caterpillars between the tropical Federal District, Brazil and temperate Minnesota, United States. Higher plant richness attributable to the presence of alternative crops within the study plots and surrounding farmland was not associated with higher predation rates in the Federal District. While the two localities had distinct predator communities as the species level, both were dominated by species from the same three taxonomic groups (Araneae, Coccinellidae, and Neuroptera) and had commensurate total predator densities. This was despite the observation that arthropod prey density and diversity was higher in the Federal District. Based on the negative effect of prey densities on artificial caterpillar predation rates it is possible that predator function was reduced in the Federal District due to predator satiation from abundant prey. While this study provides evidence to refute biogeographic arthropod predation patterns in *Brassica*-dominated agroecosystems, additional tropical-temperate comparative research is needed to identify the extent and cause of this altered pattern.

## Introduction

Biogeographic variation in biotic interaction strength is thought to create a latitudinal gradient in natural ecosystems with higher interaction strength at low latitudes (Dobzhansky 1950, Schemske et al. 2009, Moles et al. 2011). While the evidence for stronger plant defense and herbivory is mixed, studies consistently support a pattern of increased invertebrate predation at lower latitudes (Jeanne 1979, Novotny 2006, Freestone et al. 2013, Roslin et al. 2017). In natural ecosystems, stronger predation at lower latitudes may limit the dominance of any one herbivore species, facilitating greater diversity among herbivores and protecting plants from excessive herbivory (Pianka 1966, Paine 1966). In agroecosystems, arthropod predation on herbivore prey can protect crops from pest outbreaks, yet whether latitude contributes to variability in predation in these systems is unknown (Bianchi et al. 2006, Bengtsson 2015).

Ecological and biogeographic theories predict that more diverse and abundant predator communities exert stronger control on herbivore populations, yet experimental studies demonstrate variable effects of predator community characteristics on predation rates (Griffiths et al. 2008, Greenop et al. 2018). Greater densities of predators relative to prey can increase predation intensity, however the high concentration of suitable host plants in agroecosystems often favors higher densities of herbivore prey (Andow 1990, Liu et al. 2005). If prey densities outpace predator densities, predator satiation can reduce predation capacity (Samu 1993, Lampropoulos et al. 2013). Among predator taxa, interactions such as competition over shared prey and intraguild predation can inhibit the effect of predator taxonomic diversity and density on function (Rosenheim et al. 1993, Griffin et al. 2013). Predatory arthropods can become intraguild prey if they are at a vulnerable life-stage (e.g. eggs as prey, but adults as predators (Weber and Lundgren 2009)), or smaller in size (Roger et al. 2000, Sloggett 2008). Additional predator diversity would not contribute to higher predation rates on target prey if predator taxa have a propensity to interfere with one another through intraguild predation or competition. Currently, it is unclear if latitudinal diversity patterns extend to arthropod predators in agroecosystems and what impact such a pattern would have on predator functionality.

Identifying biogeographic determinants of predation strength may be crucial to meeting the world's growing demand for sustainably produced food (Katinas and Crisci 2018). Crop yields can depend on arthropod predation, yet usually only abiotic factors such as temperature, soil moisture, and light are explicitly used to make geographic recommendations to farmers (USDA-ARS 2012, Culliney 2014). The rarity of studies on biogeographic biotic interaction variation in agroecosystems likely prevents the incorporation of such factors into agricultural advice. Only one study has compared predation rates between climates in an agroecosystem, finding lower predation rates in a sub-tropical than temperate setting (Morales et al. 2018). However, this study focused on small (<0.05 hectare), diverse urban community and home gardens, which varied widely in the type of crops and associated plants grown. Whether predator dynamics in controlled agroecosystems mimics those found in small gardens or in natural ecosystems remains unexplored.

In this study the hypothesis that the biogeographic pattern in arthropod predation documented in natural ecosystems persists in agroecosystems was tested, anticipating higher predation rates in a tropical agroecosystem where greater plant and arthropod predator diversity is present. Using a single, organic-managed *Brassica oleracea* focal crop to control the agroecosystem across localities and predator exclusion cage experiments, predation intensity was measured on sentinel prey baits in a temperate and tropical locality. Whether the biotic factors associated with higher arthropod predation rates remain constant was investigated by surveying the plant and arthropod communities present in tropical and temperate *Brassica* cropping systems.

## Materials and Methods

### *Study plot selection and preparation*

Predator-exclusion cage experiments were conducted during the 2016 and 2017 growing seasons in the tropical Federal District, Brazil (16° S, 48° W), and temperate Twin Cities Metropolitan Area, Minnesota, USA (45° N, 93° W) (Table 1.1). Experiments were conducted during the main growing season for *Brassica* crops when temperatures were commensurate (Table 1.1). In the Federal District, experimental study plots were established on six organic partner farms, all of which regularly grew varieties

of *Brassica oleracea* L. (primarily collards, but also broccoli, cauliflower, and cabbage). Variation in the planned crop and non-planned weed diversity and density in these plots came from differences in farm management practices. Experimental plot dimensions ranged from 200-400 $m^2$ based on the area needed to transplant 24 experimental collard plants among existing *Brassica* plantings and plots were typically a part of a larger production field. In 2016 each partner farm hosted one study plot, whereas in 2017 each farm had two study plots separated by a minimum of 100 m. In Minnesota, two farm-sites were established at the Minnesota Agricultural Experiment Station, and one farm-site at the Rosemount Research and Outreach Center, each farm-site hosted four study plots in 2016 and 2017. In each farm-site, four collard plots were planted, separated by at least 5 m of bare ground and were 10 by 10 m in dimensions. In Minnesota, variation in weed density was controlled by variation in weed management intensity. Half of the Minnesota plots were weeded as needed to remain weed-free and half were only weeded in the 0.5 m radius around the collard plants to avoid differences in plant quality due to crop-weed competition.

In the Federal District, predator exclusion cages were 60 cm diameter by 50 cm tall. Cage covers were sewn from a white nylon organdy with a zipper installed on one side for easy access and a heavier canvas material at the bottom hem so the cage could be buried. Sham cages were of 60 cm squares of the same white mesh fabric, suspended 50 cm above the ground to allow access by predators but maintain a similar micro-climate to exclusion cages. Minnesota cage frames were 35 by 100 cm square tomato cages purchased from Burpee Seed Company and cage covers were constructed in a similar manner to those in the tropics with No-See-Um Mesh. No canvas material was added to the bottom of cage covers in Minnesota. However, sufficient additional mesh material was included in cover length (~0.25 m) to ensure proper burial.

In the Federal District, collard seedlings were purchased from a local market and grown in a mesh-enclosed greenhouse for 4-5 weeks prior to use in experiments at EMBRAPA-Cenargen, Brasília. At the start of each round of experiments 24 collard plants were transplanted randomly into existing *Brassica* plots on partner farms with half in each type of cage (predator exclusion and sham). In Minnesota, collard seedlings (variety Vates) were grown in controlled greenhouse conditions on the University of

Minnesota's St. Paul campus. Collard seedlings were transplanted to the Minnesota field plots with a Hatfield Transplanter at a row spacing of 1.25 m and plant spacing of 0.5 m. Collards grew for 5-6 weeks in the field before the start of experiments in Minnesota. Twelve collard plants were chosen in each Minnesota plot to cage, half in each type of cage (predator exclusion and sham). In both localities, experimental plants were cleaned of all arthropods except sentinel prey prior to the start of an experiment.

*Assessing predation on sentinel prey*

Experiments were initiated by inoculating the caged collard plants with several types of sentinel prey baits (Lövei and Ferrante 2017). In both localities, plants were inoculated with live aphids, lepidopteran eggs, and artificial caterpillars. In Minnesota live caterpillars were also used. Artificial caterpillars were not placed in the exclusion cages. For live caterpillar, egg, and aphid prey, a biological control service index (BSI) was calculated to quantify predation intensity (Gardiner et al. 2009). BSI scales from zero, or no biological control from predators to one, or complete control. Daily change in population of aphids was used as the prey metric of survivorship in the BSI calculation, which was $\Sigma$((aphid population change$_{exclusion}$-aphid population change$_{sham}$)/ aphid population change$_{exclusion}$)/number of exclusion cages, where aphid daily population change was calculated as (population density on the final day/population density initial day)/number of days in the trial. BSI indices for eggs and live caterpillars were calculated from prey count data at the end of the experiment with the typical BSI formula $\Sigma$((prey$_{exclusion}$-prey$_{sham}$)/ prey$_{exclusion}$)/number of exclusion cages. For the artificial caterpillars, predation rate was calculated as the proportion of prey with signs of attack divided by the total number of artificial caterpillars recovered (Howe et al. 2015). Missing artificial caterpillars were rare (0.008% of baits deployed) and they were excluded from the calculation.

During both years in the Federal District, cabbage aphids (*Brassica brevicoryne*) and turnip aphids (*Lipaphis pseudobrassicae*) colonized collard plants in the greenhouse, so before use in experiments aphids were removed leaving an initial population size of <30 individuals. Aphids were counted at the beginning of the experiment and four days later. During 2016 in Minnesota, green peach aphids (*Myzus persicae*) were obtained

from a lab colony and groups of 15 aphids were transferred to leaf clippings that were then pinned to experimental collard plants. During 2017 in Minnesota, 15 *M. persicae* were transferred from other plants in the study plots to each of the experimental plants in clip cages on a middle leaf, where they were held for two days prior to the start of the experiments. Aphids were counted at the beginning of the experiment and eight days later.

In the Federal District, *Anticarsia gemmetalis* eggs were obtained from the insect rearing facilities of EMBRAPA-Cenargen. Although this species is not typically found on *Brassica* plants, it is in the same family as *Trichopluisa ni* (Noctuidae), which is a common *Brassica* pest, and their eggs are similar in size and morphology (Capinera 2017). Eggs were laid on thick paper and sections of paper with 10 eggs were used. For two plots in 2017 sufficient numbers of eggs were not available so groups of 5 eggs were used. Two groups of eggs were pinned to the abaxial surface of a leaf through the midvein. In Minnesota, *Trichoplusia ni* and *Plutella xylostella* eggs were purchased from Benzon Research (PA). Groups of 10 *Trichoplusia ni* eggs were cut from the wax paper on which they were laid. *P. xylostella* eggs were so densely oviposited onto the foil substrate that in order to deploy standardized groups of 10 eggs, they were gently dislodged and transferred onto heavy wax paper. One group of eggs of each lepidopteran species was pinned to the abaxial surface of a leaf through the midvein in Minnesota. In both localities egg groups were collected two days after inoculation and examined under the microscope for signs of predation. Eggs were scored as survived (intact or hatched) or preyed upon (signs of chewing or sucking).

Artificial caterpillars were used during both years in Minnesota and during 2017 in the Federal District. They were made from Van Aken Plastalina Modeling Clay (Green) SKU #1839677MA, measuring 2.5 by 25mm to mimic dimensions of late-instar *Trichoplusia ni* caterpillars and were stored in 2mL centrifuge tubes in a refrigerator prior to use to avoid inadvertent impressions in the material (Muchula et al. 2019). Artificial caterpillars were affixed to the abaxial surface along the midvein with 3-4 dots of Loctite Ultra Gel Control Super Glue. In Minnesota, two artificial caterpillars were attached to each of 10 plants per plot for a total of 20 caterpillars per plot. In the Federal District, 12 artificial caterpillars were attached singly to plants for a total of 12 caterpillars per plot.

After 48 hours, caterpillars were collected from the field and kept chilled until scoring. Caterpillars were examined for signs of predation using a macro attachment to an iPhone4 camera and dissecting microscope. Supplemental Material from Low et al. (2014) (EEA DOI:10.1111/eea.12207) was used to key out attack marks to gross taxon (arthropod, bird, mammal, reptile). Two predation rates were calculated for artificial caterpillars, an arthropod predation rate, and one accounting for attacks from all taxa.

Some of the *T. ni* eggs from Benzon Research in Minnesota were reared at 30°C in plastic containers with collard leaves *ad libitum* until they reached the second instar stage. Ten larvae were transferred to each caged collard plant with a fine paintbrush and counted eight days later. During 2016 individuals were collected from the field and reared to pupation to check for parasitism. No parasitoids emerged though a few larvae succumbed to an unknown fungal pathogen.

*Characterizing plant and arthropod communities*

Plant diversity was characterized at three levels. First, plant richness and cover were surveyed in a 0.5m radius around each focal sham-caged plant. Percent cover for each plant taxon was estimated visually to the nearest 5%. Next, two 15m point-intercept transects (points dropped every 0.25m) were used to census plant richness and cover to estimate plot level plant resources (Goodall 1951). At the farm level, crop plants adjacent to study plots were surveyed to measure on-farm crop plant richness. Plants were identified to lowest taxonomic level possible (always family, often genus and species). Morphospecies richness and cover were broken down into subcategories (*Brassica* crop, weed, floral, and alternative crop).

To assess within-plot arthropod diversity, 12-24 whole *Brassica* plants and the 0.5m radius around them were surveyed by first observing all arthropods visible without disturbing vegetation and then by searching below all leaves and on the ground exhaustively. Arthropods were identified to family and characterized by functional guild (predator, herbivore-prey, pollinator) and family. Arthropod abundance of each type of guild was calculated as average per plant density within a plot. For the plots where oversampling occurred (>12 plants) 12 random survey plants were chosen to obtain a standardized sampling intensity for arthropods in all plots.

*Data Analysis*

To examine whether study plots between the two countries were comparable despite differences in plot establishment (research versus commercial farm) and management (variation in weeding intensity), plant richness and cover at the 0.5m radius, plot, and farm scale were compared. Generalized linear models of the Poisson responses of plant and floral richness were constructed to test the fixed effect of country, the nested factor of farm within country, and year. Measures of percent cover (weed, floral, *Brassica*, alternative-crop, and bare) were modeled as binomial responses of the same factors. All models were examined for overdispersion by checking the ratio of the sum squared Pearson residuals to the residual degrees of freedom; if this value was > 1.5 then a quasi-likelihood was used to correct for overdispersion. Significance of effects was tested with Wald $\chi^2$ ($\alpha = 0.05$), and MLE means were calculated using the *Anova* function in the *car* package in R (Firth et al. 2009).

To determine whether predation rates varied significantly between the two countries logistic regression models were constructed for each bait type. The binomially distributed responses of predation (BSI for eggs, aphids, and live caterpillars; arthropod and total predation rates for artificial caterpillars) were modeled as a function of the fixed effect of country, the nested factor of farm within country, and year. Model residuals were examined for signs overdispersion as described above and Wald $\chi^2$ ($\alpha = 0.05$) type II tests were run to test for significance of model parameters.

To examine whether there were systematic differences in the predator communities between the two study countries non-metric multidimensional scaling (NMDS) on counts of predator taxa was conducted. The *metaMDS* function with the *vegan* package in R was used to calculate both the dissimilarity metric and to visualize differences between localities between study countries (Oksanen et al. 2017). The Bray-Curtis dissimilarity metric was chosen as it is widely used to compare ecological communities and is robust in dealing with sparse observations (Beals 1984, Ricotta and Podani 2017). To better understand the predator diversity at the plant scale in a country, the per plant mean abundance and standard error for each predator taxa was calculated.

Finally, to understand the relationship between arthropod guilds and predation

rates, linear models were constructed to test the effect of study plot on the following predator and prey variables and the effect of these variables on predation by bait type. Variables were log transformed prior to analysis to conform with assumptions of normality. The responses of prey density (1), prey richness (2), ratio of predator to prey density (3), ratio of predator to prey richness (4), predator density (5), and predator richness (6) were modeled as a function of country, farm nested within country, and year. Next, logistic regression models of predation responses were constructed for each bait type as a function of each of the six arthropod variables, country, interaction of arthropod variable and country, farm nested within country, and year. For both arthropod variable response and predation response models, residuals were examined for signs overdispersion and Wald $\chi^2$ ($\alpha = 0.05$) were examined to determine factor significance. Averages of each arthropod variable were calculated for each country to assist in model interpretation.

**Results**

*Plant community comparison*

There were no differences in vegetation associated with the study plots between Minnesota and the Federal District as measured by the cover of the focal crop (*Brassica oleracea*), weeds, flowering plants, or bare ground (Table 1.2, S1.1). Overall plant and weed species richness were significantly higher in the Federal District than in Minnesota. One third of the farmers in the Federal District grew vegetable polycultures or practiced agroforestry, accounting for the additional plant richness (Table 1.2). These additional crops included tubers grown in patches on the edges of plots (cassava; *Manihot esculenta* and yam; *Dioscorea* sp.), shade fruit trees on plot edges or occasionally interspersed (banana, *Musa* sp.; coffee, *Coffea arabica*; and papaya, *Carica papaya*), and intercropped vegetables (beans, *Phaseolus* sp.; cucumbers, *Cucumis sativus*; green onions, *Allium* sp.; tomatoes, *Solanum lycopersicum*; and tomatillos, *Physalis philadelphica*). While these species contributed to higher plant richness, they covered at most 10% of the plot (Table 1.2). In Minnesota, farm crop richness was significantly lower than in Brazil (Table 1.2). Most adjacent land to Minnesota plots was planted to corn (*Zea mays*) and soybean (*Glycine max*) row crops, whereas in the Federal District, farmers grew a wide assortment

of other vegetable and fruit crops in adjacent fields. As weeds, focal *Brassica* crops, and bare ground accounted for ~90% of cover within study plots in both localities and the proportions of cover for each of these main plant groups was comparable, the variation in plot management and plant vegetation did not bias comparisons of arthropod predation between the localities, and if it did, it should contribute to higher predation in the Federal District.

*Predation rates*

Results from logistic regression models showed that predation rates were not significantly higher in the Federal District than in Minnesota for any sentinel bait after controlling for year and farm (Table 1.3). Predation did vary significantly among bait types, with highest predation observed on aphids and lower predation on sentinel egg and artificial caterpillars in both countries (Table 1.3, Fig. 1.1). Predation rates on live caterpillars in Minnesota were comparable to those on aphid baits, exceeding rates of predation on the artificial caterpillars (Fig. 1.1). In both localities, arthropods were responsible for the majority (87%) of attacks on artificial caterpillars. More birds (10%) than mammals (3%) attacked artificial caterpillars in Minnesota, whereas in District Federal more mammals (10%) than birds (3%) attacked artificial caterpillars.

*Arthropod communities*

The same three taxonomic groups, spiders (Araneae), coccinellid beetles (Coccinellidae), and lacewings (Neuroptera), were the most common predators in both localities (Table 1.4). Spiders were more abundant in the Federal District, whereas coccinellids were more abundant in Minnesota (Table 1.4). Lacewings ranked third in abundance in both localities. Six of the predator taxa were found in only one locality, however these were relatively rare taxa where they did occur (Table 1.4). The average per plant predator density and predator richness was similar between the two localities (Table 1.5, S1.2). Ordination by non-metric multidimensional scaling revealed distinct dissimilarities between the predator communities in the two localities (Fig. 1.2). Differences in predator communities were likely driven by higher abundances of spiders and lacewings in the Federal District versus more coccinellids in Minnesota.

In contrast to predator communities, prey were more diverse and abundant in Brazil than in the United States (Table 1.5). Prey were on average twice as dense in the Federal District than Minnesota, therefore the ratio of predators to prey was higher in Minnesota than the Federal District (Table 1.5). Aphids (*Aphididae*) and whiteflies (*Aleyrodidae*) were particularly common at high densities in the Federal District. In addition to consumptive damage caused by these hemipteran pests, a co-occurrence of fungus on plants with high hemipteran densities was observed in the Federal District. In Minnesota, aphids were common, but were not observed promoting fungal growth or in such high densities as in the Federal District (Table 1.4). While the effect of prey, predator to prey, and predator richness and density on predation rates by prey-bait type were examined, most did not have a significant impact. Only prey density had a significant negative effect on predation rates of artificial caterpillars (both all attacks and arthropod attacks), though this effect held in both countries (Table 1.6, Fig. 1.3).

**Discussion**

This study documented similar levels of predation between the Federal District, Brazil and Minnesota, United States in *Brassica* agroecosystems. By standardizing the study cropping system, the effects of geographic location separate from variation in cropping system management could be examined. Despite being located in different climactic zones, on-farm plant management led to similar amounts of *Brassica* crop and weed cover between the two localities, compared to a previous study in which plant resources varied significantly between tropic and temperate localities (Morales et al. 2018). Weed cover is an important variable to account for in agronomic comparisons as it can introduce structural complexity for arthropod predators and dilute the concentration of resources for crop specialist herbivore prey (Andow et al. 1986, Schellhorn and Sork 1997). Though the amount of weed cover did not vary between localities, weed and crop richness was significantly higher in the Federal District. Crop management has been demonstrated to impact weed richness, with organic row crops containing more diverse weed communities than conventional row crops (Menalled et al. 2001). Minnesota farm-sites, while managed organically during the experimental growing seasons, did have a previous conventional management history that could have contributed to lower weed

species richness. Despite inadequate information to discern the basis of differential weed richness between the localities, higher weed richness in the Federal District did not support higher predation rates as expected from agroecological theory (Root 1973). Federal District farmers incorporated alternative crops at low densities within *Brassica* plots in non-uniform intercropping patterns. This variation created a wide range in the type of *Brassica* to alternative crop interfaces that are not well represented by orderly experimental designs testing the effect of diversification on insect dynamics (Hooks and Johnson 2003). Additional crops in the Federal District may have provided alternative prey during times of low *Brassica* prey abundance to support predator communities, however intensive sampling on alternative crops prior to the start of experiments would have been necessary to confirm such an effect.

Though predation rates did not vary between the two localities there was variation in BSI among the types of sentinel prey. BSI for aphids was the highest among the prey types tested in both localities and BSI for live caterpillars in Minnesota was similar to BSI for aphids. BSI for egg and artificial caterpillar baits was less than half as high as BSI for aphids or live caterpillars (Fig. 1.1). Eggs in the experiments were pinned flush to the underside of *Brassica* leaves but were oviposited onto paper. It is possible that chemical or physical cues from the paper prevented arthropod predators from readily attacking eggs. Alternatively, lower predation rates on lepidopteran eggs could be attributable to the presence of preferred prey for each of the main predator groups present. Ladybird beetles were the most and second most abundant predators in Minnesota and the Federal District respectively. Though coccinellids can consume a variety of prey, those sampled in both localities were in subfamily Coccinellinae which is considered aphidophagous and therefore may have preferentially consumed aphid over egg prey (Hodek and Evans 2012). Additionally, egg age can impact rejection rates by some coccinellid species (Roger et al. 2001). Though eggs used in this study were kept refrigerated prior to slow development, high rates of hatching in the United States (mean = 0.39, SE = 0.08) may indicate that eggs were decreasing in quality for coccinellids over the course of the experiment. Consumption of *Anticarsia gemmetalis* eggs has not been well documented for either major predator groups present in the Federal District and may have been a less acceptable as prey than anticipated based on taxonomic relation to

*Brassica* pest *Trichoplusia ni* (Godfrey et al. 1989, Lowenstein et al. 2017). Spiders were the most and second most abundant predator taxa in in the Federal District and Minnesota respectively and previous studies have shown that spiders in agroecosystems have higher predation rates on caterpillars than lepidopteran eggs of the same species (Miliczky and Calkins 2002, Lowenstein et al. 2017). Further, spiders will continue to consume aphid prey at similar rates with and without access to preferred prey (Madsen et al. 2004). Mesocosm studies offering these three prey types in varying combinations to spider and coccinellid predators could clarify if prey preferences contributed to the observed lower rate of egg predation in the study.

Within Minnesota, BSI on live caterpillars was higher than on artificial caterpillars (Fig. 1.1). Two factors could have contributed to the discrepancy between predation on live versus artificial caterpillars. First, the artificial caterpillars were modeled after 4-5 instar *Trichoplusia ni* caterpillars, whereas the live caterpillars were deployed as 2$^{nd}$ instar individuals. Predation risk on *Trichoplusia ni* has been shown to decrease with body size (Roger et al. 2000). It may be that the visual size of the artificial caterpillars was sufficient to reduce predator attacks. Alternatively, reduced predation on the artificial caterpillars could be attributable to the lack of predator-recruiting chemical cues (Lövei and Ferrante 2017). The results support early research on artificial caterpillar baits, indicating this method likely represents a conservative estimate of arthropod predation (Howe et al. 2009).

Prey density, but not predator density or richness was higher in the Federal District than in Minnesota. Ladybird beetles, spiders, and lacewings dominated predator assemblages in both localities. Both spiders and coccinellids can partition space among individuals and taxa when present in multi-species assemblages to avoid interference, and can sufficiently suppress prey rates as an individual predator taxon (Weber and Lundgren 2009, Barton and Schmitz 2018, Greenop et al. 2018). Higher herbivore prey densities in the Federal District indicates that *Brassica* crops in this site experience higher pest pressure despite comparable arthropod predator function. The abundance of prey in the Federal District may have diverted predators from the sentinel prey or could have diluted the impact of predators on both existing and sentinel prey. If predators were diverted from sentinels by the high prey densities in the Federal District, predation rates may have

been underestimated. However, lower predator to prey densities throughout the surveyed plots in the Federal District also suggest a dilution effect (Ekström and Ekbom 2011, Roslin et al. 2017). A negative effect of prey density on attack rates on artificial caterpillars in both localities was observed, which suggests either a dilution or diversion effect (Fig. 1.3). More controlled prey-enrichment experiments could illuminate whether this result was due to the artificial prey type or if it is a broader phenomenon experienced by live prey.

The study suggests that trends in arthropod predation from natural areas may not hold in human managed agroecosystems. Additionally, it suggests that under similar cultivation conditions, arthropod predators may provide similar levels of pest management services regardless of geographic location. Unfortunately, if prey species are present at higher densities than predators as was observed in the Federal District, similar predation rates may be insufficient to protect tropical crop plants from pest damage. Identifying whether there is a biogeographic pattern in herbivore abundance or damage, independent from predator control variation may help identify measures that farmers can take to mitigate herbivore pest abundance. Additional comparative research is needed to understand what factors may contribute to variable rates of arthropod predation between agricultural and natural ecosystems in the same locality and whether there are management methods that can bolster predator function while minimizing herbivore population growth.

**Table 1.1.** Experimental summary information for predator-exclusion cage studies in the Federal District, Brazil and Minnesota, USA during 2016 and 2017 in *Brassica* agroecosystems.

| site | year | dates | farms | plots | cages | avg. temp (ºC) |
|---|---|---|---|---|---|---|
| Federal District | 2016 | June 2-27 | 6 | 6 | 70 | 19.86 |
| | 2017 | April 24- June 9 | 6 | 12 | 140 | 21.83 |
| Minnesota | 2016 | Sept 11-19 | 3 | 12 | 70 | 18.97 |
| | 2017 | Aug 7-15 | 3 | 12 | 70 | 19.80 |

**Table 1.2.** Plant characteristics of study *Brassica* plots in the Federal District Brazil and Minnesota, United States at the 0.5m radius, plot, and farm scales. All values show mean ± SE and statistical results are for Wald $\chi^2$ type 2 tests of main effects of country controlling for the nested factor of farm within country and year. $\chi^2$ values reported here reflect significance of main country effect on characteristic, country:farm and year effects are reported in Table S1.1 (Appendix 1). Richness values modeled as Poisson responses; cover characteristics modeled as binomial responses.

| scale | response | Federal District | Minnesota | Wald $\chi^2$ | *p* |
|---|---|---|---|---|---|
| 0.5m | Weed richness* | 3.33 ± 0.53 | 2.96 ± 0.43 | 0.40 | 0.526 |
| | Floral richness | 1.17 ± 0.26 | 0.79 ± 0.15 | 1.01 | 0.315 |
| | Weed cover (%) | 15.61 ± 5.16 | 24.84 ± 4.88 | 0.28 | 0.599 |
| | Floral cover (%) | 3.44 ± 1.08 | 9.01 ± 2.31 | 0.33 | 0.568 |
| plot | Plant richness (incl. alt-crops) | 8.44 ± 0.83 | 4.96 ± 0.46 | **19.26** | **<0.001** |
| | Floral richness | 2.50 ± 0.34 | 1.67 ± 0.19 | 3.46 | 0.063 |
| | Weed richness | 6.17 ± 0.00 | 3.96 ± 0.00 | **10.67** | **0.001** |
| | Alternative-crop richness | 1.28 ± 0.00 | - | - | - |
| | *Brassica* cover (%) | 27.40 ± 4.44 | 40.97 ± 3.04 | 0.87 | 0.351 |
| | Floral cover (%) | 16.30 ± 3.43 | 9.01 ± 2.01 | 0.53 | 0.468 |
| | Weed cover (%) | 38.61 ± 7.57 | 33.69 ± 5.77 | 0.23 | 0.628 |
| | Alternative-crop cover (%) | 0.07 ± 0.03 | - | - | - |
| | Bare (%) | 27.30 ± 5.78 | 25.34 ± 4.99 | 0.06 | 0.808 |
| farm | Crop plant richness | 11.83 ± 1.86 | 5.00 ± 0.35 | **76.77** | **<0.001** |

*quasi-likelihood to correct for overdispersion

**Table 1.3.** Analysis of deviance table for logistic regression (type 2) of effects of country (the Federal District, Brazil or Minnesota, United States), farm nested within country and year on arthropod predation by bait type. Caterpillar baits were only used in the United States, therefore only effects of year and farm were tested.

| **bait type** | **predictor** | **df** | **Wald $\chi^2$** | ***p*** |
|---|---|---|---|---|
| aphids | country | 1 | 0.21 | 0.645 |
| | year | 1 | 2.56 | 0.109 |
| | country: farm | 7 | 2.18 | 0.949 |
| eggs | country | 1 | 0.05 | 0.819 |
| | year | 1 | 0.02 | 0.903 |
| | country: farm | 7 | 1.29 | 0.989 |
| artificial caterpillars | country | 1 | 0.38 | 0.538 |
| (all attacks) | year | 1 | 0.87 | 0.351 |
| | country: farm | 7 | 0.78 | 0.998 |
| artificial caterpillars | country | 1 | 0.32 | 0.570 |
| (arthropod attacks) | year | 1 | 0.77 | 0.378 |
| | country: farm | 7 | 0.68 | 0.998 |
| caterpillar larvae | year | 2 | 0.51 | 0.775 |
| | farm | 1 | 0.05 | 0.816 |
| all baits | country | 1 | 0.27 | 0.605 |
| | bait type | 3 | **23.45** | **<0.001** |
| | year | 1 | 1.65 | 0.199 |
| | country: bait | 2 | 0.03 | 0.985 |
| | country: farm | 7 | 2.14 | 0.952 |

**Table 1.4.** Predator individuals observed per plant in study *Brassica* plots in the Federal District, Brazil and Minnesota, United States. All values show mean ± SE. Bolded lines indicate top three abundant predator taxa.

| Predator Taxa | Federal District | Minnesota |
|---|---|---|
| Anthocoridae | - | 0.035 ± 0.021 |
| **Araneae** | **1.292 ± 0.514** | **0.149 ± 0.041** |
| Asilidae | 0.028 ± 0.023 | - |
| Carabidae | 0.074 ± 0.053 | 0.035 ± 0.017 |
| Cleridae | 0.005 ± 0.005 | - |
| **Coccinellidae** | **0.894 ± 0.407** | **3.045 ± 0.515** |
| Dolichopodidae | 0.106 ± 0.036 | 0.007 ± 0.005 |
| Forficulidae | 0.009 ± 0.006 | - |
| Hymenoptera | 0.009 ± 0.009 | 0.031 ± 0.011 |
| Nabidae | - | 0.003 ± 0.003 |
| **Neuroptera** | **0.560 ± 0.180** | **0.292 ± 0.090** |
| Opilionidae | 0.005 ± 0.005 | 0.038 ± 0.017 |
| Staphylinidae | 0.074 ± 0.074 | 0.003 ± 0.003 |
| Syrphidae | 0.250 ± 0.053 | 0.017 ± 0.009 |
| Vespidae | 0.042 ± 0.032 | 0.007 ± 0.005 |
| Zygoptera | - | 0.003 ± 0.003 |

**Table 1.5.** Characteristics of the arthropod communities within study *Brassica* plots in the Federal District, Brazil and Minnesota, United States. All values show untransformed mean ± SE and statistical results are for Wald $\chi^2$ type 2 tests of main effects of country controlling for the nested factor of farm within country and year. $\chi^2$ values reported here reflect significance of main country effect on characteristic, country:farm and year effects are reported in Table S1.2. All responses except predator to prey richness responses were log transformed prior to modeling statistical tests as indicated to meet the assumptions of normality.

| **response** | **Federal District** | **Minnesota** | **Wald $\chi^2$** | ***p*** |
|---|---|---|---|---|
| prey density | 69.82 ± 24.38 | 29.02 ± 13.74 | **4.99** | **0.026** |
| prey richness | 7.00 ± 0.58 | 5.00 ± 0.59 | **5.28** | **0.022** |
| predator to prey density | 0.24 ± 0.12 | 0.99 ± 0.38 | 3.53 | 0.060 |
| predator to prey richness | 0.74 ± 0.08 | 0.83 ± 0.07 | 0.39 | 0.533 |
| predator density | 3.24 ± 0.69 | 3.31 ± 0.55 | 0.00 | 0.965 |
| predator richness | 4.83 ± 0.48 | 4.00 ± 0.51 | 2.44 | 0.119 |

**Table 1.6.** Summary of logistic regression (Wald type 2 tests) of effects of components of arthropod density and diversity, country (Federal District, Brazil; Minnesota, United States), their interaction, farm nested within country and year on arthropod predation by bait type. Caterpillar baits were only used in United States, therefore only effects of year and farm were tested.

| | | | **aphids** | | **eggs** | | **artificial caterpillar-all** | | **artificial caterpillar-arthro** | | **caterpillar** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Predictor** | **Factors** | **d.f.** | **Wald $\chi^2$** | ***p*** | **Wald $\chi^2$** | ***p*** | **Wald $\chi^2$** | ***p*** | **Wald $\chi^2$** | ***p*** | **Wald $\chi^2$** | ***p*** |
| prey density | | 1 | 0.00 | 0.980 | 0.01 | 0.933 | **7.68** | **0.006** | **9.59** | **0.002** | 0.73 | 0.393 |
| | country | 1 | 0.49 | 0.482 | 0.54 | 0.465 | 7.48 | 0.006 | 8.43 | 0.004 | | |
| | year | 1 | 8.00 | 0.005 | 0.28 | 0.595 | 12.16 | 0.000 | 13.63 | <0.001 | 0.34 | 0.559 |
| | prey density:country | 1 | 1.07 | 0.302 | 0.55 | 0.459 | 0.11 | 0.745 | 0.74 | 0.388 | | |
| | country:farm (farm caterpillar) | 7 | 5.30 | 0.623 | 12.08 | 0.098 | 9.45 | 0.222 | 12.58 | 0.830 | 3.25 | 0.197 |
| prey richness | | 1 | 0.11 | 0.740 | 0.12 | 0.726 | 0.33 | 0.568 | 0.35 | 0.556 | 0.19 | 0.659 |
| | country | 1 | 0.17 | 0.680 | 0.11 | 0.739 | 0.46 | 0.496 | 0.39 | 0.534 | | |
| | year | 1 | 2.89 | 0.089 | 0.01 | 0.910 | 0.81 | 0.369 | 0.70 | 0.401 | 0.03 | 0.856 |
| | prey richness:country | 1 | 0.52 | 0.473 | 0.00 | 0.989 | 0.00 | 0.973 | 0.03 | 0.856 | | |
| | country:farm (farm caterpillar) | 7 | 2.16 | 0.951 | 1.25 | 0.990 | 0.86 | 0.997 | 0.83 | 0.997 | 0.48 | 0.788 |
| predator to prey density | | 1 | 0.02 | 0.891 | 0.02 | 0.894 | 0.11 | 0.739 | 0.10 | 0.752 | 0.00 | 0.950 |
| | country | 1 | 0.22 | 0.643 | 0.07 | 0.786 | 0.62 | 0.429 | 0.49 | 0.483 | | |
| | year | 1 | 1.85 | 0.173 | 0.00 | 0.961 | 0.85 | 0.357 | 0.72 | 0.396 | 0.04 | 0.840 |
| | predator to prey density:country | 1 | 0.10 | 0.747 | 0.10 | 0.751 | 0.00 | 0.958 | 0.08 | 0.773 | | |
| | country:farm (farm caterpillar) | 7 | 2.03 | 0.958 | 1.30 | 0.988 | 0.58 | 0.999 | 0.51 | 0.999 | 0.48 | 0.785 |
| predator to prey richness | | 1 | 0.05 | 0.821 | 0.97 | 0.324 | 0.18 | 0.671 | 0.17 | 0.678 | 0.56 | 0.453 |
| | country | 1 | 0.17 | 0.684 | 0.16 | 0.686 | 0.50 | 0.481 | 0.38 | 0.538 | | |
| | year | 1 | 1.72 | 0.190 | 0.05 | 0.829 | 0.76 | 0.384 | 0.67 | 0.412 | 0.02 | 0.898 |
| | predator to prey richness:country | 1 | 0.06 | 0.810 | 0.06 | 0.803 | 0.01 | 0.924 | 0.07 | 0.794 | | |

| | | | aphids | | eggs | | artificial caterpillar-all | | artificial caterpillar-arthro | | caterpillar | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Wald | | Wald | | Wald | | Wald | | Wald | |
| **Predictor** | **Factors** | **d.f.** | $\chi^2$ | ***p*** | $\chi^2$ | ***p*** | $\chi^2$ | ***p*** | $\chi^2$ | ***p*** | $\chi^2$ | ***p*** |
| | country:farm (farm caterpillar) | 7 | 2.42 | 0.933 | 1.57 | 0.980 | 0.84 | 0.997 | 0.79 | 0.998 | 0.52 | 0.771 |
| predator density | | 1 | 0.59 | 0.442 | 0.63 | 0.427 | 0.25 | 0.614 | 0.09 | 0.767 | 0.02 | 0.896 |
| | country | 1 | 0.23 | 0.631 | 0.05 | 0.818 | 0.48 | 0.489 | 0.38 | 0.536 | | |
| | year | 1 | 3.08 | 0.079 | 0.01 | 0.923 | 0.98 | 0.323 | 0.83 | 0.364 | 0.04 | 0.838 |
| | predator density:country | 1 | 0.61 | 0.436 | 0.02 | 0.883 | 0.01 | 0.926 | 0.00 | 0.978 | | |
| | country:farm (farm caterpillar) | 7 | 1.97 | 0.962 | 1.34 | 0.987 | 0.69 | 0.998 | 0.60 | 0.999 | 0.50 | 0.777 |
| predator richness | | 1 | 0.00 | 0.956 | 0.29 | 0.592 | 0.00 | 0.988 | 0.01 | 0.917 | 0.03 | 0.873 |
| | country | 1 | 0.17 | 0.685 | 0.03 | 0.864 | 0.37 | 0.543 | 0.32 | 0.569 | | |
| | year | 1 | 2.84 | 0.092 | 0.07 | 0.790 | 0.86 | 0.353 | 0.75 | 0.386 | 0.06 | 0.808 |
| | predator richness:country | 1 | 0.31 | 0.578 | 0.11 | 0.735 | 0.01 | 0.903 | 0.00 | 0.977 | | |
| | country:farm (farm caterpillar) | 7 | 2.03 | 0.958 | 1.57 | 0.980 | 0.74 | 0.998 | 0.69 | 0.998 | 0.53 | 0.766 |

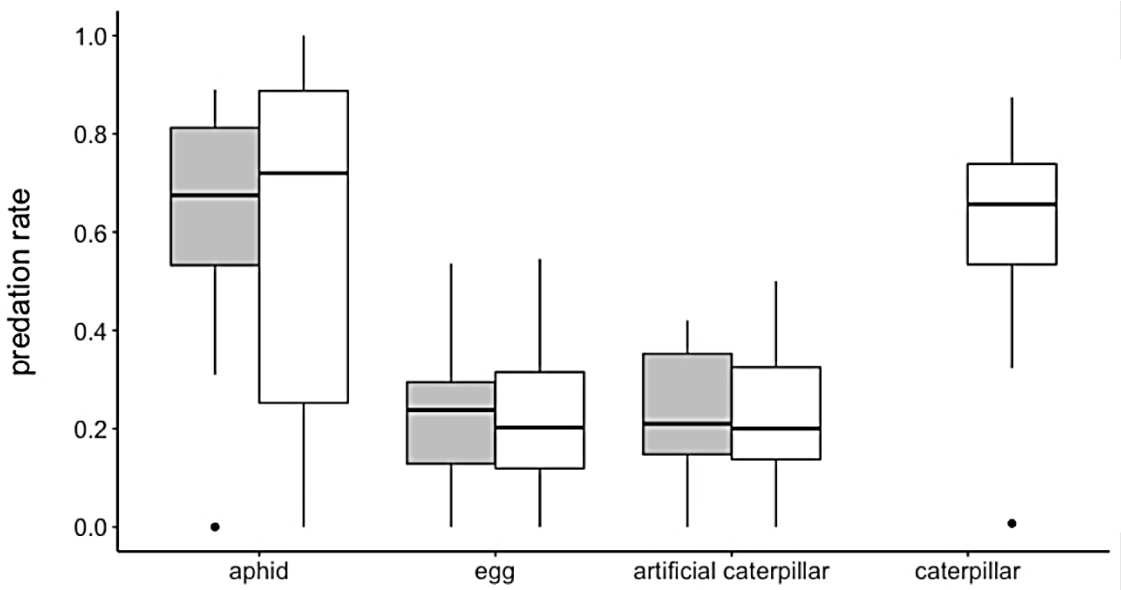



**Fig. 1.1.** Predation rates in *Brassica* agroecosystems in the Federal District, Brazil (gray) and Minnesota, United States (white) by bait type. Aphid, egg, and caterpillar predation rates calculated as biocontrol service indices (BSI, Gardiner et al. 2009). Artificial caterpillar predation rate calculated as percent recovered baits attacked (all predator types). Boxplots show medians (bold horizontal line), 25$^{th}$ and 7$^{th}$ percentiles (upper and lower box bounds), and extreme observations (bars).

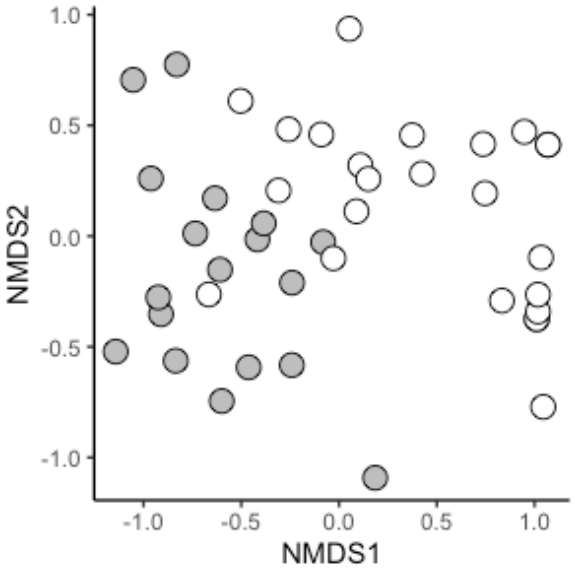



**Fig. 1.2.** Non-metric multidimensional scaling (NMDS) plot of predator taxa abundance for *Brassica* study plots within the Federal District, Brazil (gray) and Minnesota, United States (white) plots based on Bray-Curtis dissimilarity metric.

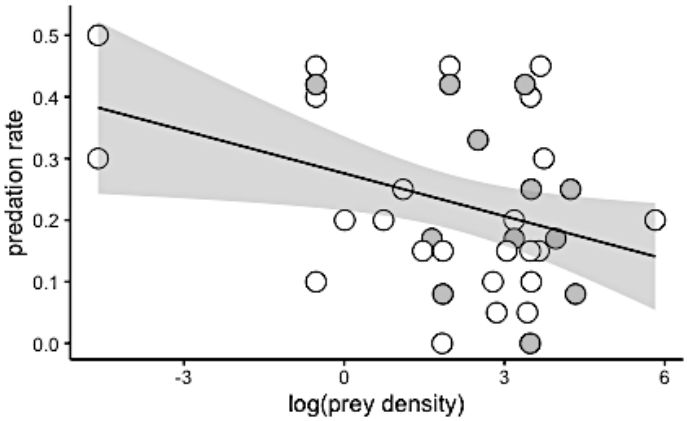



**Fig. 1.3.** Logistic regression of the prey density on predation rates of artificial caterpillars (all predator types) in the Federal District, Brazil (gray) and Minnesota, United States (white) in *Brassica* agroecosystems. Shaded area around line represents confidence interval ($\alpha = 0.05$).

## Chapter 2: Reduced arthropod predation in low latitude agroecosystems

### Summary

Arthropod predation strength on herbivore prey increases at lower latitudes in natural ecosystems, however, studies at the biogeographic scale have yet to explore such latitudinal patterns in agroecosystems. We tested the hypothesis that the latitudinal arthropod predation gradient holds in agroecosystems by assessing predation on model caterpillars in *Brassica oleracea* agroecosystems across 36º latitude in the United States and Brazil. In both countries, we found a reverse predation gradient, whereby arthropod attacks increased with latitude. However, the rate of increased arthropod predation with latitude and the associated causes of this pattern were different between the two countries, suggesting biogeographic differences in the functional organization of arthropod predation in agroecosystems.

### Introduction

In the 19th century, pioneering naturalist Alfred Russell Wallace first described biotic interaction gradients, which paralleled the latitudinal diversity gradient (Wallace 1878). Mid-20th century biologists theorized that the relatively benign, stable environment of the tropics allows for interactions to strengthen whereas at higher latitudes, harsh abiotic conditions limit interaction intensity (Dobzhansky 1950, Fischer 1960). Experimental evidence is mixed for herbivory (Moles et al. 2011, Anstett et al. 2014, Lim et al. 2015) and competition (Schemske et al. 2009, Marshall and Baltzer 2014), but solidly supports a latitudinal gradient in the rate of predation on arthropods, particularly in forest arthropod food webs (Jeanne 1979, Novotny 2006, Freestone et al. 2013, Roslin et al. 2017). In the Eastern Hemisphere, Novotny et al. (2006) found significantly higher predation on arthropods in three tropical than in three temperate forest localities. Similarly, in the Western Hemisphere, predation by ants on caterpillar prey declined with latitude across five forest localities (Jeanne 1979). Most recently, Roslin et al. (2017) examined predation on standardized model caterpillars in 22 forest localities across the world, including eight spanning North and South America, and found a distinct pattern of higher predatory attacks on model arthropods at lower latitudes.

Agroecosystems predominate the land cover of the Earth and critically impact human welfare, yet studies of large-scale latitudinal biotic interaction gradients have not

been explored in these habitats. Conversion of natural ecosystems to agriculture generally decreases species diversity (Matson et al. 1997), but the effect on biotic interaction strength, such as predation, is relatively unknown. On the one hand, the reduction in diversity might be expected to reduce predation relative to natural ecosystems, resulting in a shallower latitudinal gradient in predation strength (Andow 1991, Gurr et al. 2003). On the other hand, agroecosystems structured by cosmopolitan crop and arthropod species, may converge to have similar interaction networks regardless of location (Wilby and Thomas 2002, Bebber et al. 2014), resulting in no detectable latitudinal gradient.

As emphasized by Roslin et al. (2017), standardized methods are critical for detecting large-scale biogeographic patterns. Thus, we employed a similar, simple, standardized protocol using model caterpillars to assess latitudinal patterns in arthropod predation in agroecosystems. This method has been used in both natural and agricultural ecosystems to assess how environmental and geographic factors influence predation on lepidopteran prey (Howe et al. 2009, Molleman et al. 2016, Roslin et al. 2017, Machado et al. 2019). Additionally, we standardized the surveyed cropping system across the latitudinal gradient, evaluating predation only in organic *Brassica oleracea* L. agroecosystems. The *B. oleracea* agroecosystem has been well-researched worldwide and comprises many cosmopolitan pest caterpillars upon which we modeled the prey baits (Grzywacz et al. 2010, Furlong et al. 2013). We exposed 1100 model caterpillars in 55 sites in 9 localities originally dominated by native grassland or savanna ecosystems, across 15º north latitude in the United States and 21º south latitude in Brazil (Fig. 2.1A, Table 2.1). In addition, we characterized the plant and arthropod communities at each site, which allowed us to develop potential explanations for the latitudinal gradient in predation strength.

**Results**

Arthropods were responsible for 90% of attacks on model caterpillars across all sites (Table 2.1, Fig. 2.2). Contrary to studies in natural ecosystems, we found that the probability of arthropod attack increased significantly with latitude (Fig. 2.1B, Table 2.3) in both the United States ($\chi^2 = 53.71$, $p = 2.32 \times 10^{-13}$) and Brazil ($\chi^2 = 18.25$, $p = 1.93 \times 10^{-5}$). However, in the United States, arthropod predation increased significantly faster with

latitude than in Brazil (Table 2.3). For every 1º latitude increase in the United States, the odds of arthropod attack increased by 21% (15.3-27.6%, 95% CI) from the odds of 0.053 (0.020-0.075, 95% CI) at the lowest latitude studied (30.1º N; Austin, Texas). In Brazil, the odds of arthropod attack increased by 7.5% per 1º latitude (3.9-11.2%, 95% CI) reaching 0.724 (0.515-1.000, 95% CI) at the highest absolute latitude studied in Brazil (29.7º S; Santa Maria, Rio Grande do Sul). Neither variation in vegetation diversity (Table 2.2) or elevation (Table 2.1, 2.4) explained any of the observed predation patterns. Consequently, the differences in the intensity of the latitudinal effect suggest that the influence of agriculture on arthropod predation was quantitatively different in the United States than in Brazil.

**Discussion**

The latitudinal gradient in predation in the *B. oleracea* agroecosystem was unexpectedly reversed from that observed in to natural ecosystems (Roslin et al. 2017), so we considered potential explanations for this altered pattern. In both natural and agroecosystems predation strength is expected to be determined by some combination of absolute or relative predator species richness and/or predator abundance. Paine's Predator Hypothesis proposed that higher predator richness drives higher predation rates (Pianka 1966, Paine 1966), with some evidence in tropical agroecosystems (Togni et al. 2018). However, predator richness can also have neutral or negative effects on predation strength when predator species compete for shared prey or engage in intraguild predation (Casula et al. 2006, Griffin et al. 2013, Greenop et al. 2018). In addition, predator-prey theories suggest that predation strength is driven by the numerical abundance of predators (Wangersky 1978, Chan et al. 2017). We explored the possible explanations for the latitudinal gradients for each country, using a stepwise procedure, first to identify predator variables related to the observed predation rates (level 1), second (level 2) prey variables related to predators, third (level 3) prey resource variables related to prey, and finally (level 4) variables related to prey resources (Fig. 2.3, Table 2.5-8).

None of the explanatory variables of the reversed latitudinal gradient in predation were the same in the United States and Brazil (Fig. 2.4, Table 2.5, 2.8), indicating that the underlying causes were probably different in the two countries. In the United States, plant

richness increased with latitude and was positively associated with higher predator density and higher predation (Fig. 2.4, Table 2.5, 2.8). Additionally, higher predation rates in the United States were associated with higher predator to prey richness ratios, which increased with prey densities (Fig. 2.4, Table 2.5, 2.8). In Brazil, floral richness conformed to the classical latitudinal diversity gradient, was directly related to increased prey richness, and indirectly supported higher predator richness, which suppressed predation rates at low latitudes (Fig. 2.4, Table 2.5, 2.8).

These opposing effects of predator diversity on arthropod predation between the United States and Brazil were unexpected, although they may be related to differences in predator functional diversity that are unrelated to taxonomic diversity (Griffin et al. 2013, Greenop et al. 2018). The positive association between predation and predator to prey species richness in the United States, may indicate that the predator taxa generally exhibited niche complementarity, whereas the negative association between predation and predator species richness in Brazil may indicate that the predator taxa generally interfered with each other (Table 2.4, Fig. 2.4).

Certain predator communities were associated with stronger predation in the United States, particularly those abundant with coccinellids (Fig. 2.5, Table 2.9). Coccinellids are important predators in many agroecosystems and have exhibited a propensity to partition space and prey in order to coexist with intra and interspecific predator species (Snyder 2009, Jackson et al. 2017). Given this flexibility in function, it is not surprising that coccinellids played a role in supporting higher predation rates, particularly where increased plant richness could provide additional spatial complexity to minimize interference among predators (Finke and Denno 2002, Sicsú et al. 2015, Amaral et al. 2015).

The negative effect of predator richness on predation rates could not be attributed to coccinellids or any other predator community in Brazil (Fig. 2.5). Predator diversity can reduce predation if predator species interfere with each other (Snyder and Wise 1999). Spiders were the most commonly observed predator taxon and the only taxa observed in four of the five sites in Brazil with the strongest predation. Spiders were likely strongly suppressed by known intraguild ground beetle (Carabidae) predators, as when both taxa cooccurred predation rates fell by 30% in one of the localities, Minas

Gerais (Dinter 1998). In the District Federal, the predator taxa included the active generalists (Asilidae, Dolichopodidae, Vespidae) and primarily aphidophagous species (Coccinellid, Chrysopidae, and Syrphidae larvae), which may have interfered with each other (Snyder and Wise 1999, Greenop et al. 2018).

These findings suggest that arthropod herbivore prey may experience reduced mortality from arthropod predators in lower latitude agroecosystems. Conditions for arthropod herbivores are already favorable at low latitudes in agriculture, where abundant plant resources and warm climates promote population growth over a longer growing season (Bebber et al. 2014). Greater herbivore pressure at lower latitudes has been documented indirectly through global patterns of maize and rice losses to insects and national records of insecticide use on soybean within the United States (Ziska 2014, Deutsch et al. 2018). Yet, no direct biogeographic study of herbivore pressure has been undertaken in agroecosystems to mirror the numerous latitudinal studies of herbivory in natural ecosystems (Moles et al. 2011). Increased herbivory could elicit higher plant defenses in crops in low latitude agriculture, including the production of predator recruiting volatiles (Poelman et al. 2012, Züst et al. 2012). Yet, if plant defenses contributed to predator function, we would have expected to see predation rates and densities conform to those observed in natural ecosystems rather than the inverse predation gradient observed in this study.

This study highlights persistent knowledge gaps in harnessing arthropod predators for pest control services as an alternative to chemical controls. Evidence for different underlying causes of the reverse latitudinal pattern in predation between the United States and Brazil raises the question of whether agroecological recommendations developed based on biotic interactions observed in one region will transfer effectively to another region. These results indicate that farmers in the United States could improve pest control by increasing predator abundance, whereas if Brazilian farmers followed the same advice they could reduce pest control in the absence of the right combination of predator taxa (Fig. 2.4). Global cultivation of crop plants such as *B. oleracea* may have resulted in a homogenization of the many of associated arthropod herbivore species, but differences in overall biotic communities and landscape contexts persist. Additional investigation is needed to understand how the functional organization of arthropod predation varies by

region and latitude and how to use the knowledge to increase the capacity of predators to control agricultural pests.

## Materials and Methods

### *Site Selection*

We assessed predation rates in *Brassica oleracea* agroecosystems across latitudinal transects in central United States (30°- 45°N) and eastern Brazil (8°- 30°S) during the 2017 growing season (Table 2.1). We concentrated on the *B. oleracea* system because a similar suite of cosmopolitan herbivore pest species attacks *Brassica* crops in both countries (Bonnemaison 1965, Philips et al. 2014, Holtz 2015). We selected study sites in 5 locations in the United States and 4 locations in Brazil. In each location, study sites were embedded in similar background native ecosystems (grasslands and savannas) with access to a metropolitan area market to minimize between-site and region variations. All sites were located on practicing organic farms, which had no pesticide applications that could interfere with arthropod predator function (Gentz et al. 2010, Bommarco et al. 2011). Study sites within each locality in both countries spanned a range of within-field vegetational diversity, to explore how vegetational diversity found among agroecosystems may affect the latitudinal pattern in arthropod predation. We found no significant effect of plant species richness on arthropod predation within locality, indicating that the variation in plant diversity did not obscure latitudinal patterns (Table 2.2). In the United States we selected five localities: southeast Minnesota (45°N), central Iowa (41°N), eastern Kansas (40°N), central Oklahoma (36°N), and central Texas (30°N). In Brazil we selected four localities: central Rio Grande do Sul (30°S), eastern Minas Gerais (20° S), the Federal District (16°S) and eastern Pernambuco (8°S). Elevational differences among localities were less pronounced in the United States (range = 275m) than in Brazil (range = 1188m) where selecting localities spanning a range of latitudes necessitated traversing the elevated central plateau of the country (Table 2.1). In each locality, we established 4-12 study sites in *Brassica* production fields, each site at least 10x20 m. Plants at all sites were in the mid- to late-vegetative growth stage (Welch and Harwood 2014) (Table 2.1). In total, predation rates in 55 *Brassica* sites were assessed with an average of 6.11 (sd = 2.67) sites per locality. This compares favorably

with previous studies, which assessed predation at 2 (Jeanne 1979), 3 (Novotny 2006), or 5 (Hardwick et al. 2017) sampling sites per locality, and 5 (Jeanne 1979) or 6 (Novotny 2006) localities, or 8 (Roslin et al. 2017) localities in North and South America.

***Survey Assessment Methods***

We followed methods used by Roslin et al. (2017), described by Howe et al. (2009) and reviewed by Lövei and Ferrante (2017) for hand-rolling cylindrical 0.5 x 3 cm green plasticine artificial caterpillars (Van Aken Plastalina 10508). These dimensions were chosen to mimic mid to late-instar *Trichoplusia ni* (L.) larvae, an important lepidopteran *Brassica* pest in both study countries (Root 1973, Oliveira et al. 2013). The use of these model caterpillars has been shown to provide accurate relative estimates of arthropod predator activity (Tvardikova and Novotny 2012, Ferrante et al. 2014, Molleman et al. 2016). At each study site an average of 18.25 (sd = 3.33) caterpillar baits were glued with Loctite™ Control Superglue to the underside central vein of a lower leaf, the most common location we encountered late-instar lepidopteran larvae (Tvardikova and Novotny 2012). Baits were exposed for two days, as in Roslin et al. (2017), collected into 2ml centrifuge tubes, and stored on ice until scoring to avoid any non-predator indentations in the malleable plasticine material. We used the scoring key developed by Low et al. (2014) to score caterpillars as not-attacked or attacked by mammals, birds, or arthropods (Fig. 2.2). Missing caterpillar baits (n = 31) were removed from the analysis as we could not confirm that absence was due to predation. Overall mean recovery rate of baits was 96.9 ± 7% (Table 2.1).

On the day we deployed the artificial caterpillars, we surveyed the plant and arthropod communities in the plot. We used the point-intercept method to characterize the plant community (Goodall 1951). We established two 20 m transects, recorded all plants intercepted by points every 0.5m along those transects, and calculated percent cover for *B. oleracea* and all non-*Brassica* plants as well as species richness of non-*Brassica* plants. We also identified which plant taxa had flowers with nectar and/or pollen at the time of sampling. To characterize the arthropod community, we sampled 12-24 *Brassica* plants and the other plants and the ground in a 0.5 m radius around the focal plant. We simplified ant counts to presence/absence on a plant, to take into account

recruitment behavior, and expressed this as the proportion of plants with ants. Arthropods were identified in the field to family or species when possible. Arthropod community data were rarified to account for unequal sample sizes among sites. We calculated the density and taxonomic richness of all arthropods present, as well as for subgroups of arthropod predators and the ratio of predator to prey density and richness for each site. Densities of coccinellids, spiders, and parasitoids were calculated as subgroups of predators.

***Data Analysis***

*Latitudinal patterns in arthropod predation*

We used logistic regression to test whether arthropod predation depended on latitude (logit link, binomial error) using country as a factor and absolute latitude of each site as a continuous variable. With country, latitude and their interaction in the model, we could determine simultaneously if there is a latitudinal pattern and if it differs for Brazil and the United States. The Pearson goodness-of-fit statistic indicated no significant overdispersion ($\chi^2 = 58.87$, $df = 51$, $p = 0.2097$), and the full model rejected the global null hypothesis (Beta = 0; likelihood ratio $\chi^2 = 335.58$, $df = 4$, $p = 2.28 \times 10^{-71}$). We found that predation increased with latitude in both the United States and Brazil (Table 2.3), opposite the prediction that predation is higher at lower latitudes. Arthropod predation increased more strongly with latitude in the United States than Brazil (Table 2.3). Given the range of elevation observed in Brazil (1183 m), we used logistic regression to test if arthropod predation also depended upon elevation in Brazil logit link, quasibinomial error to correct for overdispersion: Pearson $\chi^2 = 43.54$, $df = 24$, $p = 8.62 \times 10^{-3}$). While the full model rejected the global null hypothesis (Beta=0; likelihood ratio $\chi^2 = 75.56$, $p = 9.03 \times 10^{-10}$), elevation did not significantly influence arthropod predation (Table 2.4).

Consequently, we explored the predictors associated with the latitudinal gradient separately for each country. We used a stepwise procedure first to identify predator predictors directly related to the observed predation rates and then examined (level 2) prey predictors of predators, then (level 3) prey resource predictors of prey and finally (level 4) predictors of prey resources (Fig 2.3). We stopped the procedure when no

significant predictor was found or when latitude was the only significant predictor. We preferred this approach to path analysis, as we did not believe a single path could be specified as a reasonable *a priori* hypothesis.

*Direct predictors of arthropod predation (Level 1)*

Paine's biogeographic predator hypothesis suggests that predation will be higher when there is: 1) higher predator species richness, 2) higher predator to prey species richness, 3) and a higher proportion of predator taxa in a community (Pianka 1966, Paine 1966). In addition, classical ecology theories suggest that higher predation occurs with: 4) higher predator density, 5) higher ratio of predator to prey densities, and 6) higher proportion predacious individuals in a community (Wangersky 1978, Chan et al. 2017). We used these 6 continuous predator variables as predictors of arthropod predation in each country in the first level of the analysis. As these predictors were not independent (*e.g.*, predator density versus predator to prey density), we could not test for multicollinearity using variance inflation factors (VIF). We used univariate models to test for the effect of each individual predator variable on arthropod predation with a quasibinomial error structure to correct for overdispersion in the dataset. We corrected for multiple hypothesis testing with the Ryan-Holm stepdown Bonferroni procedure ($\alpha$ = 0.05) to identify which predator variables were significant predictors of arthropod predation in each country.

Higher predator density and a higher ratio of predator to prey species richness were statistically significant predictors of higher predation rates in the USA data (Table 2.5), consistent with a part of Paine's predator hypothesis and classical ecological theory. In contrast, higher predator species richness was a significant predictor of reduced predation rates in the Brazil data (Table 2.5), contrary to predictions.

*Predictors indirectly associated with arthropod predation (Levels 2-4)*

Next, in level 2 of the analysis, we examined predictors that might influence the significant direct predictors of predation rates in each country (Table. 2.5). For predator density, we examined the influence of prey density, prey richness, plant richness, non-crop cover, floral cover, and floral richness. For predator to prey richness we examined

the same factors with the exception of prey richness and with the addition of *Brassica*-cover as a prey resource and latitude as a factor in species diversity. For predator richness, we examined all of the listed predictors for predator density with the addition of the latitude predictor (Tables 2.5-2.8). The reasoning for selecting these potential predictors of each predator predictor follows. Higher prey density can increase predator density (Holling 1961, Chang and Kareiva 1999) and increase predator richness by reducing competition among predator taxa (Cardinale et al. 2006). Similarly, higher prey species richness can increase predator richness or density via resource partitioning (Casula et al. 2006). Higher plant and floral richness, and cover of non-crops and flowering plants can increase predator richness or density as many predators rely on the resources provided by diverse plant resources (Blaauw and Isaacs 2014, Gurr et al. 2017). Higher *Brassica* cover also can increase prey richness and density by providing more prey resources (Root and Cappuccino 1992, Schellhorn and Sork 1997) and account for high variability considering the differences across sites. Finally, latitude can influence predator or prey richness following the documented latitudinal gradients in abundance and richness in natural ecosystems (Pianka 1966, Willig et al. 2003).

First, we needed to remove multicollinearity among the predictors for each model. We used variance inflation factor scores (VIF) to assess the relative contribution of each predictor to multicollinearity using multiple regression (Yamashita et al. 2007, Dormann et al. 2013) (Table 2.6). When a predictor had a VIF score exceeding 5, which indicates multicollinearity, we removed that single predictor, the model was refit and we assessed whether the modification resolved collinearity among remaining variables (Table 2.6). If more than one predictor had a VIF exceeding 5 we removed the predictor judged to be less biologically relevant for a given response. For example, the VIF scores of both floral cover and floral richness exceeded 5 in the model for predator density in the United States, and we kept floral cover judging that the amount of floral resources was more likely related to predator density than the species richness of flowering plants (Rebek et al. 2005, Otoshi et al. 2015). In all cases removal of one predictor resolved multicollinearity among predictors (Table 2.6).

A multiple linear regression of the remaining predictors was evaluated with stepwise (forward and backward) regression adding or dropping predictors based on $AIC_c$

(Yamashita et al. 2007). In all cases the forward and backward methods resulted in the same best model. The best model was checked for overdispersion and homogeneity of error variance and was compared to the null model (Table 2.7). Parameters in the best model were based on extracted coefficients and SE estimated by maximum likelihood estimates (Zuur and Ieno 2016) (Table 2.8). Significance of the predictor on the response variables was determined with Wald $\chi^2$ type 3 test of main effects.

Significant predictors from this level 2 analysis were then analyzed in a similar fashion (VIF scores, stepwise multiple regression) to determine the significance of level 3 predictors. The significant level 2 predictors in the United States were plant richness for predator density and prey density for predator to prey richness (Table 2.8). In Brazil, both prey richness and latitude significantly predicted predator richness (Table 2.8). In the level 3 analysis with the United States data we tested for the influence of non-crop cover and latitude on plant richness, and plant richness, *B. oleracea* cover, non-crop cover, floral cover, and floral richness on prey density. For the Brazilian data we tested for the influence of plant richness, *B. oleracea* cover, non-crop cover, floral cover, floral richness, and latitude on prey richness. We selected these predictors in level 3 of the analysis based on the following reasoning. Plant richness in natural ecosystems increases at lower latitudes and could follow a similar pattern in the managed agroecosystems given that non-managed weed species contribute to overall diversity (Barthlott et al. 2007). Increasing the area covered by non-crop plants may also increase plant species richness given the demonstrated species-area relationship in agronomic weed communities (Pollnac et al. 2009). Prey richness and density are likely enhanced by the concentration and diversity of host plant resources, however whether overall plant richness, crop, and non-crop cover contributes to the host resources will depend on the host ranges of the prey species present (Andow 1991, Schellhorn and Sork 1997). The presence of floral resources in agroecosystems can increase oviposition by lepidopteran adults and increase subsequent caterpillar prey populations and support the abundance of floral feeding prey groups (Zhao et al. 1992). Finally we expect that the diversity, but not density of arthropod prey species may follow the latitudinal species diversity gradient (Andrew and Hughes 2005).

From this level 3 analysis, we found in the United States that greater plant richness was significantly predicted by latitude while no factor significantly predicted prey density (Table 2.8). In Brazil, floral richness was the only significant predictor of prey richness (Table 2.8). In a final level 4 analysis we tested potentially important predictors of floral richness in Brazil, namely *B. oleracea* cover, non-crop cover and latitude. Flowering plants in agroecosystems may follow the latitudinal pattern in species diversity, particularly when richness comprises primarily native weeds rather than managed crops (Jansson and Davies 2008). Furthermore, floral richness may follow the species-area relationship, increasing with the amount of area devoted to non-crop cover versus crop plants. Latitude was the only significant predictor of floral richness in this model (Table 2.8).

In these analyses, a single predictor could occur at more than one level of the analysis. For example, non-crop cover can be associated with the diversity and abundance of prey (and used in level 2 analyses), with important alternative resources for prey (such as shelter and food, and used in level 3 analyses), or with floral plant richness (and used in level 4 analyses). In no case was a single predictor significant at more than one level of the analysis except for latitude. We used the significant predictors to construct a network of interactions that could account for the observed latitudinal gradient in predation (Fig. 2.4)

*Predator community assessment*

We performed an ordination analysis to understand if differences in predation rates among sites were related to predator community composition, not simply density or richness. First we used non-metric multidimensional scaling (NMDS) to visualize differences in predator communities, using the Bray-Curtis dissimilarity metric on counts of taxa in study localities with the *metaMDS* function in the *vegan* package in R (Oksanen et al. 2017). NMDS with the Bray-Curtis metric is widely applied for comparisons among communities due to its robustness and effectiveness especially when dealing with sparse data matrices (Beals 1984, Faith et al. 1987, Ricotta and Podani 2017). We then fit the environmental response vector of arthropod predation rate (number of baits attacked/number of baits recovered per site) with the *envfit* function using 999

permutations. We visualized the results of the environmental vector analysis in *ggplot* in R, with the vector of arthropod predation rate pointing in the direction of most rapid change and the length of the arrow proportional to the strength of the gradient (Figure 2.5) (Oksanen 2015). We assessed goodness of fit of the vector of arthropod predation and the ordination of predator communities with Pearson's correlation coefficient.

The analysis showed that arthropod predation rate correlated significantly with the ordinated NMDS dissimilarities among predator communities in the United States ($r^2$ = 0.25, $p$ = 0.021), but not in Brazil ($r^2$=0.03, $p$ = 0.725). This finding, together with the result of stronger arthropod predation where predators were abundant and diverse relative to prey indicated that the predator communities in the United States may exhibit niche complementarity giving rise to overall higher predator function (Casula et al. 2006). Furthermore, in the United States there may be a certain group of predators that contribute strongly to this pattern. In Brazil, where predator richness was found to decrease predator function, the lack of correlation between predator community composition and predation rates may indicate a pattern of predator interference among species and individuals (Casula et al. 2006).

To further investigate if a specific predator groups played a significant role in the relationship between predator community composition and predation rates in the United States we used logistic regression to test for the additive influence of coccinellid, spider, and parasitoid density on arthropod predation. Multicollinearity among the three types of predator densities was low (all VIF <2), and we tested for significant effects of the predator groups with the Wald $\chi^2$ type III test (Table 2.9). We found that coccinellid density was a significant predictor of arthropod predation in the United States. All data analysis was carried out in R version 3.5.1 (R Core Team 2018).

**Table 2.1.** Summary of artificial caterpillar monitoring methods, recovery rates, and attacks by predator type for each study site.

| | | | | | | | | | | count of bait attacks | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **country** | **state[1]** | **site** | **latitude** | **longitude** | **elevation (MASL)** | **start** | **end** | **baits (n)** | **recovery %** | **arthropod** | **bird** | **lizard** | **mammal** | **all** |
| **Brazil** | **RS** | 1 | -29.72 | -53.72 | 96 | 3-Sep | 5-Sep | 20 | 90 | 8 | 0 | 0 | 0 | 8 |
| | | 2 | -29.72 | -53.72 | 96 | 3-Sep | 5-Sep | 20 | 100 | 5 | 0 | 0 | 0 | 5 |
| | | 3 | -29.72 | -53.72 | 96 | 3-Sep | 5-Sep | 20 | 100 | 10 | 0 | 0 | 0 | 10 |
| | | 4 | -29.62 | -53.69 | 102 | 4-Sep | 6-Sep | 20 | 95 | 6 | 0 | 0 | 0 | 6 |
| | | 5 | -29.62 | -53.69 | 102 | 4-Sep | 6-Sep | 20 | 100 | 4 | 0 | 0 | 0 | 4 |
| | | 6 | -29.62 | -53.69 | 102 | 4-Sep | 6-Sep | 20 | 100 | 10 | 0 | 0 | 0 | 10 |
| | **MG** | 1 | -20.70 | -42.80 | 805 | 30-Aug | 1-Sep | 20 | 90 | 8 | 0 | 0 | 2 | 10 |
| | | 2 | -20.70 | -42.80 | 805 | 30-Aug | 1-Sep | 20 | 75 | 5 | 0 | 0 | 1 | 6 |
| | | 3 | -20.70 | -42.80 | 805 | 30-Aug | 1-Sep | 20 | 65 | 4 | 0 | 0 | 1 | 5 |
| | | 4 | -20.70 | -42.80 | 805 | 30-Aug | 1-Sep | 20 | 80 | 8 | 0 | 0 | 1 | 9 |
| | | 5 | -20.70 | -42.80 | 805 | 1-Sep | 3-Sep | 20 | 80 | 6 | 0 | 0 | 0 | 6 |
| | | 6 | -20.70 | -42.80 | 805 | 1-Sep | 3-Sep | 20 | 95 | 9 | 0 | 0 | 0 | 9 |
| | **FD** | 1 | -16.03 | -47.77 | 864 | 29-May | 2-Jun | 12 | 100 | 2 | 0 | 0 | 0 | 2 |
| | | 2 | -16.03 | -47.77 | 864 | 27-Aug | 31-Aug | 12 | 91.67 | 3 | 0 | 0 | 0 | 3 |
| | | 3 | -15.61 | -48.08 | 1279 | 27-Feb | 3-Mar | 12 | 100 | 3 | 1 | 1 | 1 | 6 |
| | | 4 | -15.61 | -48.08 | 1279 | 5-Jun | 9-Jun | 12 | 100 | 5 | 0 | 0 | 0 | 5 |
| | | 5 | -15.66 | -48.11 | 1174 | 22-May | 26-May | 12 | 100 | 1 | 0 | 0 | 0 | 1 |

| | | | | | | | | | | count of bait attacks | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **country** | **state[1]** | **site** | **latitude** | **longitude** | **elevation (MASL)** | **start** | **end** | **baits (n)** | **recovery %** | **arthropod** | **bird** | **lizard** | **mammal** | **all** |
| | | 6 | -15.66 | -48.11 | 1174 | 12-Jun | 24-Aug | 12 | 100 | 2 | 0 | 0 | 0 | 2 |
| | | 7 | -15.99 | -47.85 | 1104 | 29-May | 2-Jun | 12 | 100 | 3 | 0 | 0 | 2 | 5 |
| | | 8 | -15.99 | -47.85 | 1104 | 27-Aug | 31-Aug | 12 | 100 | 1 | 0 | 0 | 0 | 1 |
| | | 9 | -15.56 | -48.03 | 1177 | 27-Feb | 3-Mar | 12 | 100 | 2 | 0 | 0 | 0 | 2 |
| | | 10 | -15.56 | -48.03 | 1177 | 5-Jun | 9-Jun | 12 | 100 | 2 | 0 | 0 | 0 | 2 |
| | | 11 | -15.65 | -48.20 | 1146 | 22-May | 26-May | 12 | 100 | 4 | 0 | 0 | 0 | 4 |
| | | 12 | -15.65 | -48.20 | 1146 | 12-Jun | 24-Aug | 12 | 100 | 3 | 0 | 0 | 0 | 3 |
| | **PE** | 1 | -8.26 | -35.50 | 471 | 4-Sep | 6-Sep | 20 | 90 | 0 | 0 | 0 | 0 | 0 |
| | | 2 | -8.26 | -35.50 | 471 | 4-Sep | 6-Sep | 20 | 100 | 0 | 0 | 0 | 0 | 0 |
| **USA** | **TX** | 1 | 30.26 | -97.70 | 138 | 1-Jul | 3-Jul | 20 | 100 | 0 | 1 | 0 | 0 | 1 |
| | | 2 | 30.26 | -97.70 | 138 | 30-Jun | 30-Jun | 20 | 100 | 1 | 0 | 0 | 0 | 1 |
| | | 3 | 30.26 | -97.70 | 138 | 30-Jun | 30-Jun | 20 | 100 | 0 | 1 | 0 | 0 | 1 |
| | | 4 | 30.09 | -97.84 | 212 | 30-Jun | 2-Jul | 20 | 100 | 0 | 0 | 0 | 0 | 0 |
| | | 5 | 30.19 | -97.52 | 122 | 1-Jul | 3-Jul | 20 | 100 | 2 | 0 | 0 | 0 | 2 |
| | | 6 | 30.26 | -97.48 | 164 | 1-Jul | 3-Jul | 20 | 100 | 1 | 0 | 0 | 0 | 1 |
| | **OK** | 1 | 35.50 | -97.53 | 364 | 24-Jun | 26-Jun | 20 | 100 | 1 | 1 | 0 | 0 | 2 |
| | | 2 | 35.50 | -97.53 | 364 | 24-Jun | 26-Jun | 20 | 100 | 1 | 0 | 0 | 0 | 1 |
| | | 3 | 35.67 | -97.50 | 357 | 24-Jun | 26-Jun | 20 | 100 | 5 | 0 | 0 | 0 | 5 |
| | | 4 | 35.56 | -97.47 | 349 | 24-Jun | 26-Jun | 20 | 100 | 2 | 0 | 0 | 0 | 2 |
| | **KS** | 1 | 38.98 | -95.22 | 252 | 7-Apr | 9-Apr | 20 | 100 | 6 | 0 | 0 | 0 | 6 |
| | | 2 | 38.98 | -95.22 | 252 | 7-Apr | 9-Apr | 20 | 100 | 8 | 0 | 0 | 0 | 8 |
| | | 3 | 38.96 | -95.21 | 257 | 7-Apr | 9-Apr | 20 | 100 | 5 | 0 | 0 | 0 | 5 |

| | | | | | | | | | | count of bait attacks | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **country** | **state[1]** | **site** | **latitude** | **longitude** | **elevation (MASL)** | **start** | **end** | **baits (n)** | **recovery %** | **arthropod** | **bird** | **lizard** | **mammal** | **all** |
| | | 4 | 38.96 | -95.21 | 257 | 8-Apr | 10-Apr | 20 | 100 | 4 | 0 | 0 | 0 | 4 |
| | | 5 | 39.10 | -95.04 | 255 | 8-Apr | 10-Apr | 20 | 100 | 6 | 0 | 0 | 0 | 6 |
| | | 6 | 38.93 | -95.54 | 334 | 9-Apr | 19-Jun | 20 | 100 | 2 | 0 | 0 | 0 | 2 |
| | | 7 | 39.26 | -94.97 | 320 | 9-Apr | 19-Jun | 20 | 100 | 2 | 0 | 0 | 0 | 2 |
| | **IA** | 1 | 41.77 | -92.72 | 307 | 1-Apr | 3-Apr | 20 | 100 | 6 | 2 | 0 | 0 | 8 |
| | | 2 | 41.76 | -92.71 | 310 | 31-Mar | 2-Apr | 20 | 100 | 6 | 0 | 0 | 0 | 6 |
| | | 3 | 41.16 | -93.20 | 296 | 1-Apr | 3-Apr | 20 | 100 | 7 | 1 | 0 | 0 | 8 |
| | | 4 | 41.48 | -95.05 | 391 | 1-Apr | 3-Apr | 20 | 100 | 5 | 1 | 0 | 0 | 6 |
| | | 5 | 41.76 | -93.81 | 294 | 2-Apr | 4-Apr | 20 | 95 | 7 | 0 | 0 | 0 | 7 |
| | | 6 | 41.67 | -93.74 | 272 | 2-Apr | 4-Apr | 20 | 95 | 7 | 0 | 0 | 0 | 7 |
| | **MN** | 1 | 45.00 | -93.17 | 295 | 24-Mar | 26-Mar | 20 | 100 | 9 | 1 | 0 | 0 | 10 |
| | | 2 | 44.99 | -93.17 | 289 | 24-Mar | 26-Mar | 20 | 100 | 11 | 0 | 0 | 0 | 11 |
| | | 3 | 45.23 | -92.73 | 314 | 25-Mar | 27-Mar | 20 | 100 | 6 | 4 | 0 | 0 | 10 |
| | | 4 | 45.63 | -93.44 | 299 | 25-Mar | 27-Mar | 20 | 100 | 10 | 3 | 0 | 0 | 13 |
| | | 5 | 44.71 | -93.10 | 285 | 25-Mar | 27-Mar | 20 | 100 | 5 | 1 | 0 | 1 | 7 |
| | | 6 | 45.07 | -93.85 | 284 | 26-Mar | 28-Mar | 20 | 100 | 10 | 0 | 0 | 0 | 10 |
| | | | | | | | **totals** | **1004** | **97** | **249** | **17** | **1** | **9** | **276** |

[1]State abbreviations are as follows: Rio Grande do Sul (RS), Minas Gerias (MG), Federal District (DF), Pernambuco (PE), Texas (TX), Oklahoma (OK), Kansas (KS), Iowa (IA), and Minnesota (MN)

**Table 2.2.** Analysis of deviance table for logistic regression (type 3) on arthropod predation to test whether selecting for a range of plant diversity within a locality has the potential to obscure latitudinal patterns.

| **Effect** | ***df*** | **Wald $\chi^2$** | ***p*** |
|---|---|---|---|
| Locality | 8 | 46.1 | 2.32E-07 |
| Locality * plant richness | 8 | 9.84 | 0.2765 |

**Table 2.3.** Analysis of deviance table for logistic regression (type 3) of country and latitude on arthropod predation and maximum likelihood parameter estimates (MLE) with significance tests and 95% confidence limits.

| Effect | *df* | Wald $\chi^2$ | *p* |
|---|---|---|---|
| Country | 2 | 107.18 | $5.31 \times 10^{-24}$ |
| Latitude*country | 2 | 71.96 | $2.36 \times 10^{-16}$ |

| Parameter | *df* | MLE | Wald $\chi^2$ | *p* | Profile likelihood 95% Confidence Limits | |
|---|---|---|---|---|---|---|
| Brazil intercept | 1 | -2.419 | 39.76 | $2.88 \times 10^{-10}$ | -3.192 | -1.686 |
| USA intercept | 1 | -8.767 | 67.43 | $2.19 \times 10^{-16}$ | -10.956 | -6.763 |
| Brazil slope | 1 | 0.072 | 18.25 | $1.93 \times 10^{-5}$ | 0.039 | 0.106 |
| USA slope | 1 | 0.191 | 53.71 | $2.32 \times 10^{-13}$ | 0.142 | 0.244 |

**Table 2.4.** Analysis of deviance table for logistic regression (type 3) of elevation on arthropod predation in Brazil.

| Effect | *df* | Wald $\chi^2$ | *p* |
|---|---|---|---|
| Elevation | 1 | 1.480 | $2.24 \times 10^{-1}$ |

| Parameter | *df* | MLE | Wald $\chi^2$ | *p* | Profile likelihood 95% Confidence Limits | |
|---|---|---|---|---|---|---|
| Intercept | 1 | -0.623 | 4.057 | 0.030 | -1.165 | -0.103 |
| Slope | 1 | -0.001 | 1.127 | 0.236 | -0.001 | 0.000 |

**Table 2.5.** Estimated maximum likelihood estimates (MLE) of the slopes of univariate regressions of the effect of potentially important predictor variables on arthropod predation including standard errors, Wald $\chi^2$, and $p$ values adjusted by the Ryan-Holm stepdown Bonferroni procedure with $\alpha$ = experiment-wise error rate of 0.05. Significant models are bolded.

| country | level | response | variable | df | MLE | SE | $\chi^2$ | $p$ |
|---|---|---|---|---|---|---|---|---|
| USA | 1 | arthropod predation | predator richness | 1 | 0.286 | 0.14 | 4.10 | 1.23E-01 |
| | | | **predator density** | **1** | **0.264** | **0.10** | **7.49** | **6.19E-03** |
| | | | **predator:prey richness** | **1** | **0.743** | **0.33** | **4.99** | **5.04E-02** |
| | | | predator:prey density | 1 | 0.330 | 0.17 | 3.87 | 1.83E-01 |
| | | | proportion of predator taxa | 1 | 1.327 | 1.19 | 1.24 | 8.43E-01 |
| | | | proportion of predator individuals | 1 | 1.139 | 0.76 | 2.24 | 5.15E-01 |
| Brazil | 1 | arthropod predation | **predator richness** | **1** | **-0.163** | **0.07** | **4.82** | **2.81E-02** |
| | | | predator density | 1 | -0.174 | 0.15 | 1.27 | 6.99E-01 |
| | | | predator:prey richness | 1 | -0.812 | 0.43 | 3.53 | 1.71E-01 |
| | | | predator:prey density | 1 | -0.856 | 1.09 | 0.62 | 9.66E-01 |
| | | | proportion of predator taxa | 1 | -2.218 | 1.03 | 4.66 | 6.06E-02 |
| | | | proportion of predator individuals | 1 | -1.568 | 1.57 | 0.99 | 8.53E-01 |

**Table 2.6.** Variance inflation factors for potential predictor variables of sig. predator responses by level of analysis. Bolded variables indicate high multicollinearity (VIF > 5). Final VIF values post-removal of variables contributing most to multicollinearity.

| country | level | response | variables | VIF | final VIF |
|---|---|---|---|---|---|
| USA | 2 | Predator density | prey density | 1.25 | 1.24 |
| | | | prey richness | 1.19 | 1.11 |
| | | | plant richness | 2.40 | 2.36 |
| | | | non-crop cover | 1.60 | 1.60 |
| | | | floral plant richness | **24.46** | - |
| | | | floral plant cover | **24.68** | 1.84 |
| | 2 | Predator to prey richness | prey density | 1.27 | 1.24 |
| | | | plant richness | 2.43 | 2.43 |
| | | | crop cover | 2.29 | 2.19 |
| | | | non-crop cover | 2.13 | 2.13 |
| | | | floral cover | **22.78** | - |
| | | | floral richness | **22.16** | 1.80 |
| | | | latitude | 2.20 | 1.80 |
| | 3 | Plant richness | non-crop cover | 1.08 | 1.08 |
| | | | latitude | 1.08 | 1.08 |
| | 3 | Prey density | plant richness | 2.02 | 1.95 |
| | | | crop cover | 1.33 | 1.25 |
| | | | non-crop cover | 1.46 | 1.46 |
| | | | floral cover | **21.72** | 1.67 |
| | | | floral richness | **22.55** | - |
| Brazil | 2 | Predator richness | prey density | 3.30 | 1.69 |
| | | | prey richness | 6.68 | 1.93 |
| | | | plant richness | 3.82 | 1.68 |
| | | | non-crop cover | 1.26 | 1.24 |
| | | | floral cover | **11.21** | - |
| | | | floral richness | 1.88 | 1.76 |
| | | | latitude | 2.46 | 1.44 |
| | 3 | Prey richness | plant richness | 3.71 | 3.71 |
| | | | crop cover | 1.93 | 1.93 |
| | | | non-crop cover | 2.11 | 2.11 |
| | | | floral cover | 1.45 | 1.45 |
| | | | floral richness | 4.42 | 4.42 |
| | | | latitude | 1.67 | 1.67 |
| | 4 | Floral richness | crop cover | 1.92 | 1.92 |
| | | | non-crop cover | 1.91 | 1.91 |
| | | | latitude | 1.01 | 1.01 |

**Table 2.7.** Significance and goodness of fit for generalized multiple regression models by stepwise $AIC_c$ (backward and forward). Full model is the initial model for selection after removing multicollinear variables (Table 2.6).

| country | level | response | model | $\chi^2$ | *p* | adj.$R^2$ | $AIC_c$ |
|---|---|---|---|---|---|---|---|
| USA | 2 | Predator density | full | 6.95 | 2.25E-01 | 0.04 | 117.07 |
| | | | best | 4.40 | 3.60E-02 | 0.11 | 107.27 |
| | 2 | Predator to prey richness | full | 14.14 | 2.81E-02 | 0.22 | 46.95 |
| | | | best | 12.84 | 1.21E-02 | 0.25 | 40.87 |
| | 3 | Plant richness | full | 5.61 | 6.04E-02 | 0.18 | 119.80 |
| | | | best | 3.99 | 4.58E-02 | 0.14 | 118.92 |
| | 3 | Prey density | full | 6.12 | 1.90E-01 | 0.06 | 282.48 |
| | | | best | 3.29 | 6.99E-02 | 0.07 | 276.46 |
| Brazil | 2 | Predator richness | full | 18.72 | 4.67E-03 | 0.50 | 104.64 |
| | | | best | 16.10 | 3.19E-04 | 0.44 | 93.46 |
| | 3 | Prey richness | full | 8.51 | 2.03E-01 | 0.49 | 110.09 |
| | | | best | 7.57 | 2.27E-02 | 0.50 | 97.23 |
| | 4 | Floral richness | full | 9.51 | 2.32E-02 | 0.18 | 100.21 |
| | | | best | 9.42 | 8.99E-03 | 0.22 | 97.40 |

**Table 2.8.** Summary of the parameter estimates from the best multivariate models of the significant direct predictors of arthropod predation (level 2), and the best multivariate models of the significant indirect predictors (level 3-4) showing maximum likelihood estimates (MLE) of predictor slopes, standard errors, Wald $\chi^2$, and associated *p* values for type 3 tests.

| country | level | response | variable | MLE | SE | $\chi^2$ | *p* |
|---|---|---|---|---|---|---|---|
| USA | 2 | Predator density | **plant richness** | **0.298** | **0.14** | **4.40** | **3.59E-02** |
| | 2 | Predator to prey richness | **prey density** | **0.008** | **0.00** | **7.61** | **5.82E-03** |
| | | | crop cover | -0.725 | 0.38 | 3.69 | 5.47E-02 |
| | | | floral richness | -0.155 | 0.10 | 2.65 | 1.03E-01 |
| | | | latitude | 0.031 | 0.02 | 2.98 | 8.41E-02 |
| | 3 | Plant richness | **latitude** | **0.035** | **0.02** | **3.86** | **4.96E-02** |
| | 3 | Prey density | plant richness | 4.722 | 2.62 | 3.24 | 7.19E-02 |
| Brazil | 2 | Predator richness | **prey richness** | **0.182** | **0.11** | **8.08** | **4.49E-03** |
| | | | **latitude** | **-0.054** | **0.02** | **6.41** | **1.13E-02** |
| | 3 | Prey richness | floral cover | -7.253 | 4.69 | 2.39 | 1.22E-01 |
| | | | **floral richness** | 0.134 | 0.05 | **7.70** | **5.53E-03** |
| | 4 | Floral richness | non-crop cover | 0.763 | 0.52 | 2.18 | 1.40E-01 |
| | | | **latitude** | **-0.047** | **0.02** | **6.64** | **9.95E-03** |

**Table 2.9.** Summary of parameter estimates from multivariate regression of predator group densities on arthropod predation rate including VIF scores, maximum likelihood estimates (MLE) of predictor slopes, standard errors, Wald $\chi^2$, and associated $p$ values for type III test.

| response | country | variable | VIF | MLE | SE | df | $\chi^2$ | $p$ |
|---|---|---|---|---|---|---|---|---|
| arthropod predation | United States | **coccinellid density** | **1.11** | **0.330** | **0.073** | **1** | **20.57** | **5.75E-06** |
| | | spider density | 1.20 | -1.029 | 0.790 | 1 | 1.69 | 1.93E-01 |
| | | parasitoid density | 1.31 | 0.003 | 0.007 | 1 | 0.24 | 6.23E-01 |

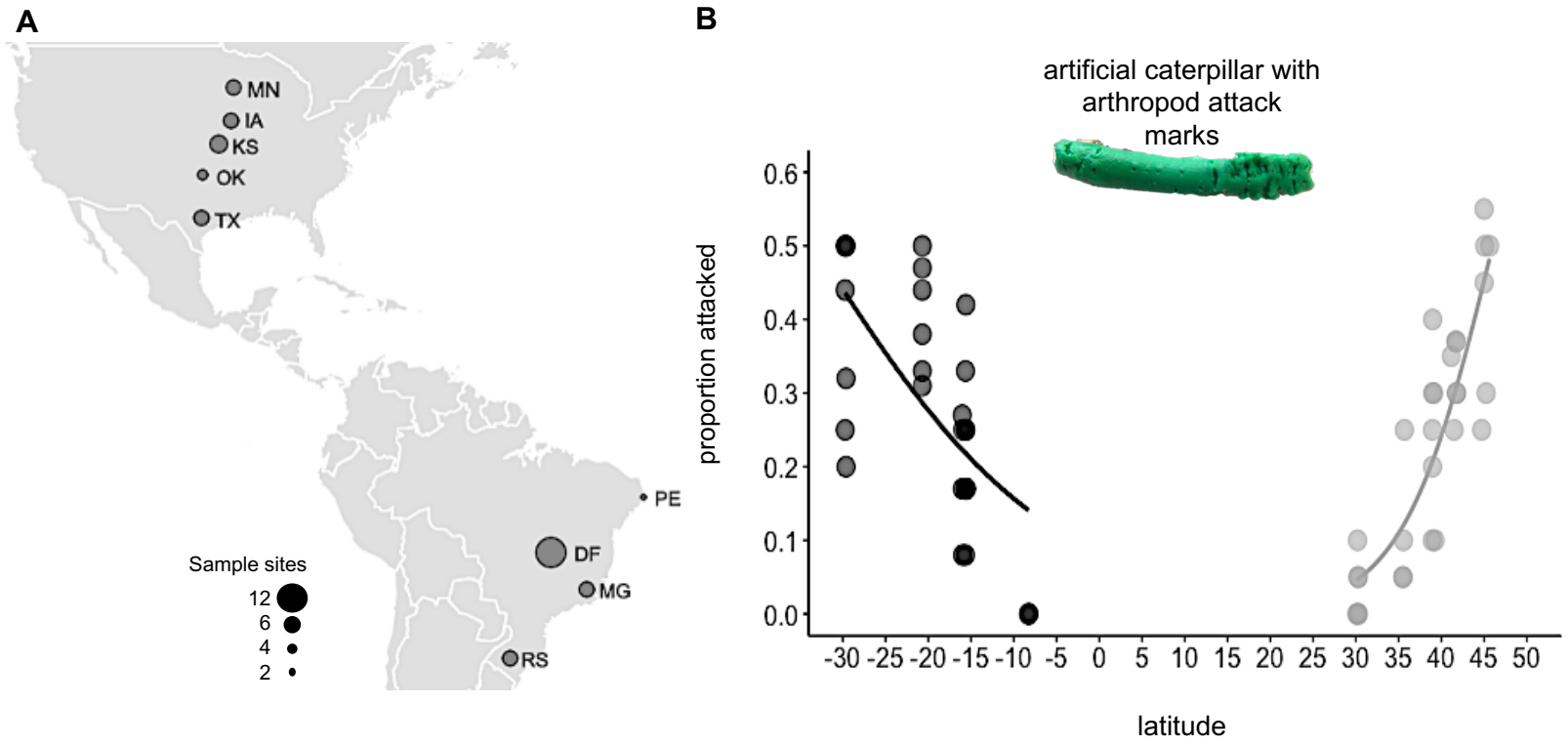



**Fig. 2.1.** Map of study locations by locality with scatter plot showing the predation rate on model caterpillars by latitude for each study site. Size of locality marker scales to number of sample sites per state. Arthropod predation rate as the proportion of attacked model caterpillars by latitude in Brazil (black) and USA (gray) *Brassica oleracea* agroecosystems. Data points are partially transparent and appear darker when overlapping. Curves are the fitted equations from logistic regression.

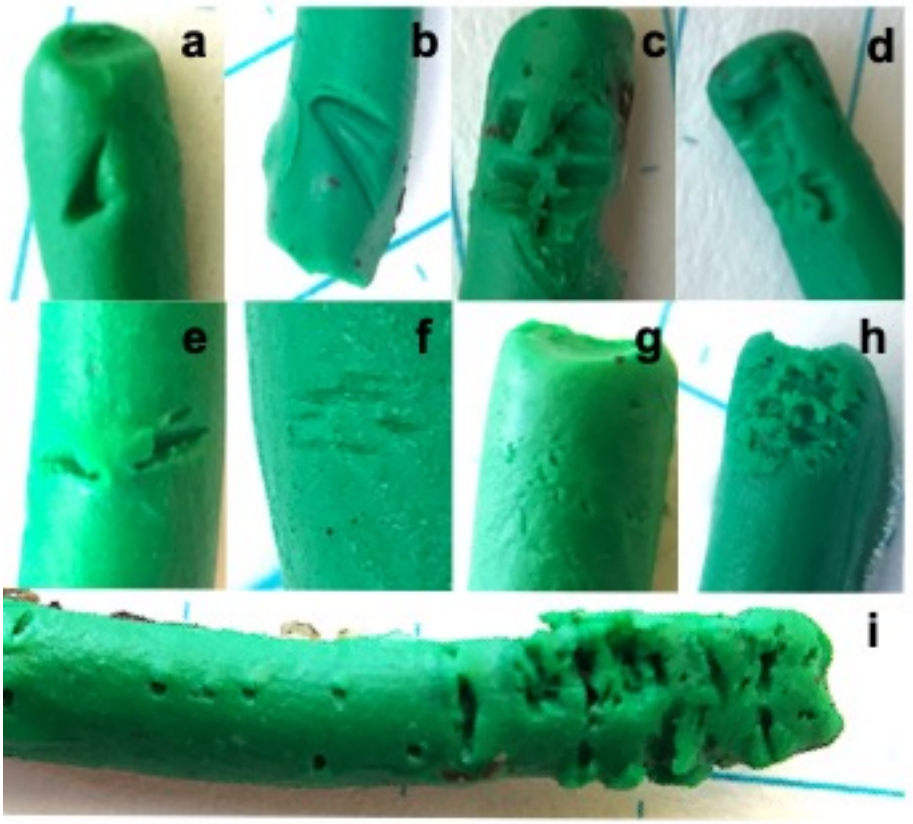
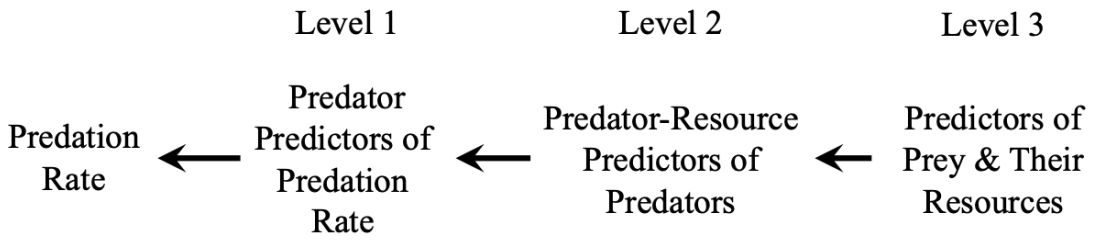



**Fig. 2.2.** Example of bite marks of birds (a, b), mammals (c, d), arthropods (e-i) on model caterpillar baits.

**Fig. 2.3.** Iterative stepwise procedure to identify predictors associated with the observed latitudinal gradient in predation rates.



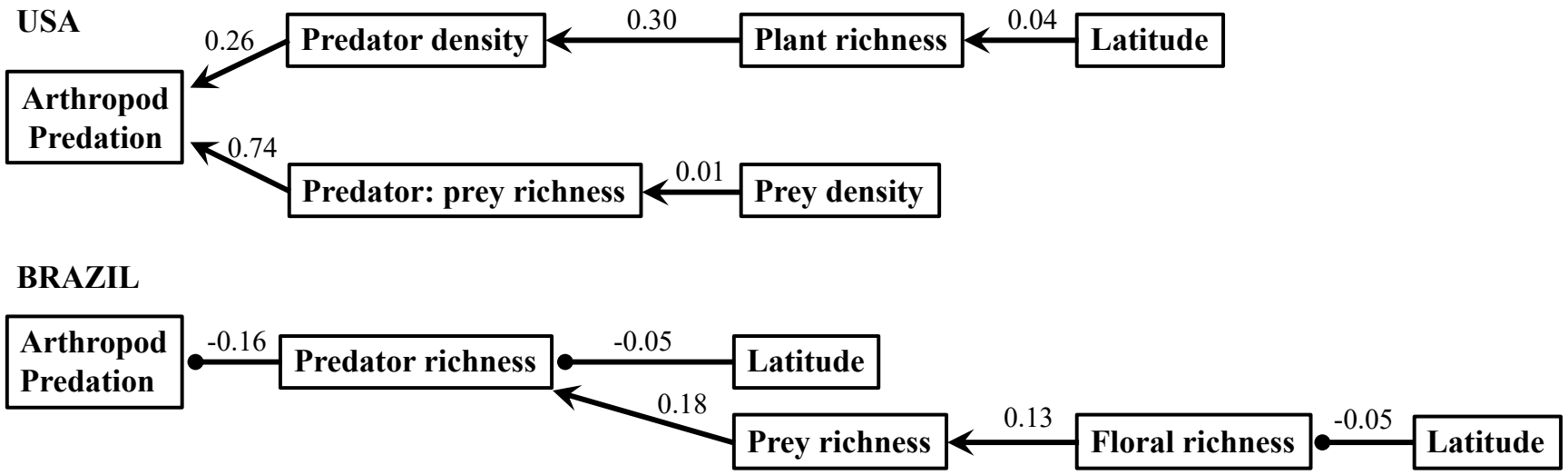



**Fig. 2.4.** Interaction network affecting latitudinal gradient of arthropod predation. Lines ending with circles are negative associations; arrowheads indicate positive associations. Numbers indicate maximum likelihood estimates of association.

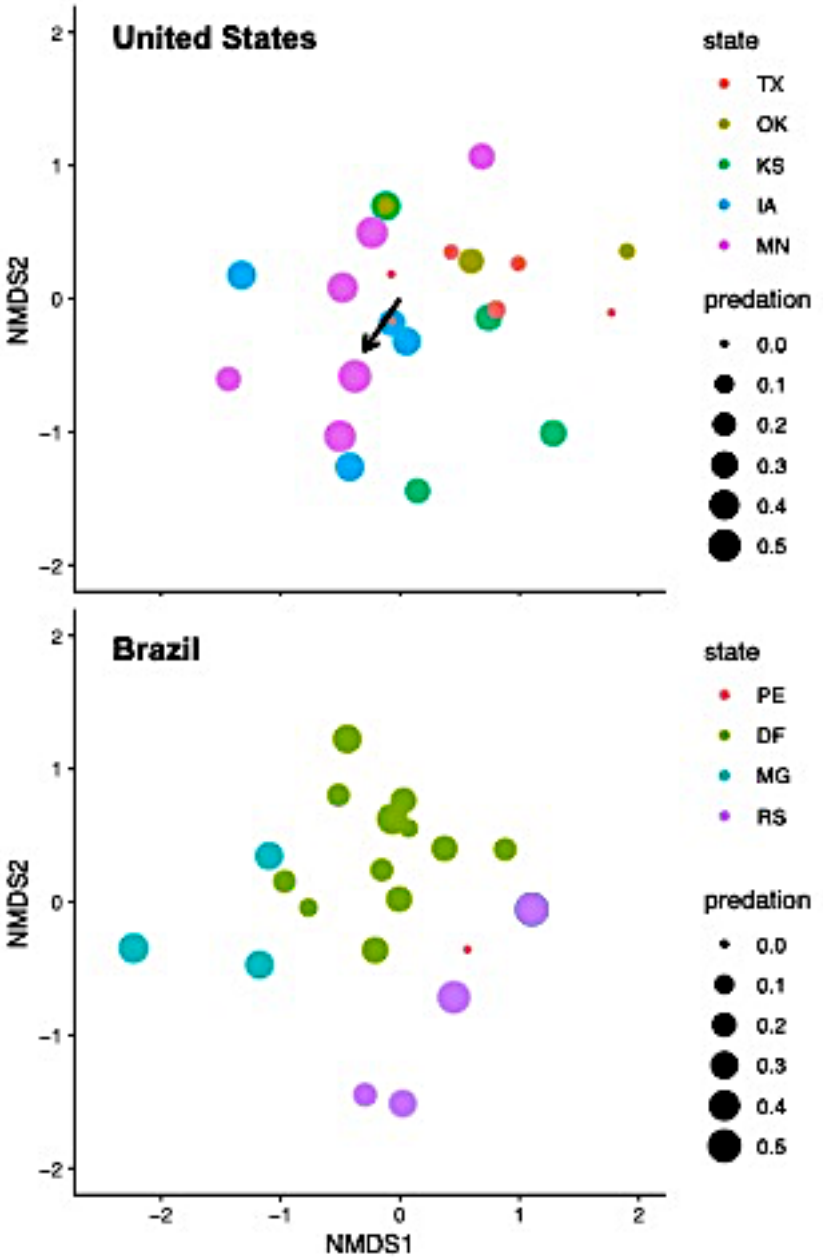



**Fig. 2.5.** Non-metric multidimensional scaling (NMDS) plot of predator taxa abundance for study sites by country using the Bray-Curtis dissimilarity metric. Arrow length in United States panel represents significant ($\alpha = 0.05$) correlation between NMDS and arthropod predation ($r^2 = 0.25$, $p = 0.021$) and is oriented towards the direction of most rapid change in predation. Predation rate was not significantly correlated with NMDS in Brazil ($r^2=0.03$, $p = 0.725$).

## Chapter 3: Independent verification of metabarcoding hits improves diet breadth analysis of arthropod predators in an agroecosystem


### Summary

In agroecosystems, entomophagous arthropod predators can provide critical pest management services, yet consumption of non-target prey may reduce the level of predation service provided. Metabarcoding provides new opportunities for studying prey consumption by entomophagous arthropods, but its wider application in pest management faces several technical challenges. This study explores whether new metabarcoding primers, designed, tailored for entomophagous arthropods in agroecosystems can improve prey detection and coverage compared with published metabarcoding primers. Bioinformatic pipelines for unclustered and clustered reads were tested, and reliability of prey detection was verified by downstream species-specific melting curve analysis of the original prey DNA community present in the guts of three species of coccinellid beetles. Results show that the in-house primers performed similarly in detection of prey taxa as previous primers. Read clustering increased rates of true and false positive prey detection. False positive and false negative prey detections were not efficiently minimized by the use of multiple metabarcoding primers or by the use of a particular bioinformatic pipeline. Reliance on metabarcoding read abundances as a cut off to infer true-positive prey detection was not supported by this study. As the metabarcoding primer and bioinformatic pipeline choice did not fully preclude false positive and false negative prey detections, the validation of the metabarcoding results should not be neglected and should be especially addressed in the most sensitive applications in ecology.


### Introduction

Identifying the diet breadth of a predator is a fundamental, yet often difficult step in establishing its role in an environment and its effect on prey species (Jiang and Morin 2005, Bianchi et al. 2009, Crowder and Snyder 2010). Advances in high throughput sequencing (HTS) now enable researchers to efficiently identify minute amounts of consumed prey DNA in the gut of a predator rather than relying upon lab feeding assays or field observations focused on species of concern (Furlong 2015). Additionally, HTS

techniques can detect previously unknown prey and characterize the diet breadth of an individual or population (Pompanon et al. 2012, Shehzad et al. 2012, Deiner et al. 2017). Metabarcoding, whereby universal primers are used to amplify barcode-markers from many different taxa within a mixed DNA sample, has proven to be an effective method for molecular diet analysis (Kaunisto et al. 2017, McInnes et al. 2017, Galan et al. 2018, Eitzinger et al. 2019). However, to ensure reliable metabarcoding results, care must be taken to avoid the accumulation of potential errors from PCR primer bias, platform-specific sequencing errors, and bioinformatic workflow flaws (Coissac et al. 2012, Corse et al. 2017, Leray and Knowlton 2017, Jusino et al. 2019). Addressing these sources of error is crucial in applied ecological settings, where the specific species comprising the diet of a predator can determine its capacity to manage pest populations.

In agricultural ecosystems, entomophagous arthropod predators are known to play an important role in controlling potential pest populations, but less is known about predator consumption of non-pest prey (Furlong and Zalucki 2010, Paula et al. 2016). Consumption of diverse prey can enhance efficacy in controlling pests, if it promotes persistence in the environment, or it can reduce efficacy if they are diverted to consume non-pest prey (Harmon and Andow 2004). Metabarcoding of gut-contents promises improved understanding of prey consumption. However, standard barcode markers developed for species-level identification, such as the Folmer region of cytochrome oxidase I (*cox1*), require amplification of a 658 bp fragment which may not be possible to recover from degraded DNA in predator guts, with modal fragment sizes of < 200 bp (Deagle et al. 2006). Yet, the wide use of the Folmer region has generated rich databases that can be employed to design shorter barcodes for specific diet applications. The necessity to identify a region of optimal variation (i.e., sufficiently low intraspecies and high interspecies variation) increases with shorter barcodes as fewer base-pairs provide information to distinguish between species. Additionally, these markers need to be flanked by highly conserved regions of DNA for primers to bind with similar efficiency for a broad array of prey taxa. Primers designed to amplify shorter barcodes of mixed arthropod samples have been developed for freshwater-bulk arthropod samples and arthropods common in bat diets (Zeale et al. 2011, Vamos et al. 2017). In agroecosystems, it remains unknown whether these primers can adequately identify prey

of entomophagous arthropods, as the design excluded or deprioritized reference sequences from major Hemipteran or Coleopteran prey groups.

After sequencing, data processing using standard bioinformatic pipelines could negatively affect the accuracy of detections if they inappropriately eliminate valid, but rare prey items, or retain false-positive prey. For example, detection rates can be distorted by differential prey species digestibility (Alberdi et al. 2019). Sequence read counts are likely biased towards less-digested prey and cannot be accurately used to quantify the relative abundance of sample components as is common in bulk sample analysis (Deagle et al. 2018). Even when gut-content data are only interpreted as presence/absence data, read abundances are often used as detection threshold criteria, thereby influencing results. Read abundance is also employed in the read clustering algorithms used to form operational taxonomic units (OTUs). Clustering is commonly applied in metabarcoding pipelines to reduce redundancy in matching sequences to a reference database, to account for amplification biases, and to avoid false-positive hits from the expected platform specific sequencing errors (Porter and Hajibabaei 2018). However, if barcodes are not sufficiently variable among related taxa, rare reads can get absorbed into OTUs dominated by abundant reads, creating false-negatives (Flynn et al. 2015). Ideally, clustering parameters should be set to balance the benefit of reducing false-positives with the risk of increasing false-negatives (Leray et al. 2013, Gueuning et al. 2019). Yet rarely do studies compare the relative risks between clustered and unclustered bioinformatic approaches.

An alternative to relying upon read abundances to determine the validity of metabarcoding hits is to conduct an independent species-specific PCR assay. Unlike bulk-sample analysis, where the high diversity within a sample could limit the feasibility of conducting a secondary PCR validation step, diet-samples are likely to be less diverse, especially in simplified agroecosystems. Detection by both metabarcoding and a second diagnostic assay that specifically targets a potential prey species has stronger support as a true prey item than metabarcoding alone. Furthermore, a molecular validation step allows researchers to identify false negatives by testing for the presence of potential prey items that were not detected by metabarcoding but are known to be present in the environment.

In this study we analyzed gut-contents of adult coccinellid beetles collected from *Brassica* agroecosystems to assess methods aimed at improving the molecular gut-content analysis of entomophagous arthropods. We tested the impact of choices made at the PCR, bioinformatic, and interpretation steps of a metabarcoding study in order to test if (i) targeting taxa in the primer design process increases taxonomic coverage and sensitivity over more generic primers, (ii) OTU clustering methods reduce false positives as compared to read based identifications, and (iii) read abundance assures true-positive prey detection, if set against an independent validation step using species-specific primers.

## Materials and Methods

*Metabarcode primer design and selection*

To detect prey in the entomophagous coccinellid beetles, widely used primer-pairs in the mitochondrial barcode regions of the cytochrome c oxidase subunit 1 (*cox1*) and 16S ribosomal *(rrnL)* genes were tested (Table 3.1). These primers were chosen based on frequency of use in studies aimed at detecting arthropods. However, these primers were not designed to detect all of the most common prey groups consumed by arthropod predators in agroecosystems and supplemental species-specific primers have been required to detect important prey species (Gomez-Polo et al. 2016). To improve metabarcoding methods for arthropod predator diet analyses in agroecosystems the general pipeline developed by Elbrecht and Leese (2017) was implemented to design and test new *cox1* barcode primers as follows.

For arthropod taxa present in agroecosystems, the package PrimerMiner (v0.15) was used to batch download all available DNA barcode *cox1* and mitogenome sequences from the NCBI and BOLD databases (1/21/17). This package dereplicated and clustered (3% dissimilarity) sequences into OTUs to avoid overrepresenting well sequenced taxa in the primer design process. In total, information from 18 arthropod orders encompassing 182 families was downloaded (Table 3.2, S3.1). Mitogenomes from each order were aligned in Geneious v7.1.9 (http://www.geneious.com/) with the MAFFT alignment and the consensus sequence was extracted with a 25% threshold to retain some sequence variability (Kearse et al. 2012). *Cox1* sequences were then mapped onto the extracted

consensus sequence for each order. Due to the low number of mitochondrial sequences available for Strepsiptera (*n*=4) and Thysanoptera (*n*=7) to form a consensus sequence for an order, the *cox1* sequences were mapped to consensus sequence from the respective sister groups. Coleoptera mito-consensus was used as the consensus for Strepsiptera and the Hemiptera mito-consensus was used for Thysanoptera (Johnson et al. 2018).

The Folmer Region fragment was extracted from the *cox1* sequences by mapping the LCO and HCO primers amplifying this region to the consensus sequence. To remove remaining gaps in the alignment, the strip alignment tool (removing 1% gaps at a time) was used to condense the alignment down to 709 bp retaining the primer binding regions. In the alignments with fewer than 1% of reference sequences covering the LCO primer binding sites, the sequences were extracted prior to the strip alignment command to avoid removing the LCO binding site, aligned separately and then realigned with the gap-removed main alignment (Katoh and Standley 2013, Elbrecht and Leese 2017b). All order-specific *cox1* sequence alignments were visualized in one alignment plot with the *plot_alignments* function from the PrimerMiner package in R. This function color codes the proportion of base-pairs (A, C, G, or T) at each position along the alignment by order and generates a consensus sequence across all orders.

To identify regions of high variability for potential primers, DegePrime (Hugerth et al. 2014) was used to generate all possible degenerate (d=1-576) primers (18-24 bp) with as high coverage as possible along all windows of the combined alignment of target orders. For each window along the alignment, DegePrime also generated measures of entropy, calculated as $-\Sigma P_i \log(P_i)$, where $P_i$ is the frequency of oligomer i. Coverage of each primer at a given position in the Folmer region was plotted by its length and separately by its level of degeneracy (Fig S3.1a,b). Level of entropy by position was also plotted (Fig S3.1c). From the graphs of entropy, regions of high variability and low variability were identified to target the primer design which was complemented with visual inspection of the PrimerMiner plotted alignments for regions of high GC content.

Primer-pair agroF1 and agroR1 was developed to amplify a minibarcode (119 bp). AgroR1 primer is a modified frame-shifted reverse of primer BF1 (Vamos et al. 2017). Primer agroR2 was designed to amplify a region of high entropy with BF1 and has a higher GC% than BR1 for the target prey taxa in this study; and, therefore, should

amplify with greater efficiency (Krehenwinkel et al. 2017). Degeneracies were added to the agroF1/R1 and agroR2 primers with the aid of the sequence alignment plot to accommodate as much variation at each position as possible. In an attempt to detect plants directly or indirectly consumed by the predators, a primer-pair for a barcode region in the internal transcribed spacers (ITS) of the nuclear ribosomal RNA gene cluster was used (Cheng et al. 2016). All primer-pairs designed in this work and by others are presented in Table 3.1. Location of the *cox1* primers in the Folmer region are illustrated in Fig. 3.1.

*DNA extraction*

Ladybird beetles from three species, *Harmonia axyridis* (n = 21), *Hippodamia convergens* (n = 20) and *Coleomegilla maculata* (n = 20) were collected from two research plots of collards (*Brassica oleracea*) at the Minnesota Agricultural Experimental Research Station in St. Paul, Minnesota, USA on August 8$^{th}$, 2017. Collected beetles were stored in 95% ethanol at -5°C prior to DNA extraction. Specimens were rinsed in ultrapure MilliQ water and individually transferred to 2 ml screw cap vials with two zinc-plated 4.5 mm beads inside. Each individual and two control-blanks (no sample added) received 100 µL DNA binding buffer from the Monarch PCR & DNA Cleanup Kit (New England Biolabs, NEB), 12 mAU proteinase K, and 2 mg/ml RNase A. Whole beetle samples were then homogenized at 4 m/s for 20 s with a MP FastPrep®-24 (MP Biomedicals Inc.). Beads were removed from the samples using a magnetic bar and samples were centrifuged at 10,000x*g* for 2 min at 25°C before being incubated at 56°C for 2 h in a water bath. The remaining DNA extraction procedure was followed as indicated in the kit manual. DNA samples and blanks were stored at -20°C when not immediately used. Individual coccinellid DNA was pooled by species and research plot to create six pooled samples of about 10 beetles each for metabarcoding: T1) *Harmonia axyridis* sampled in plot 1; T2) *Coleomegilla maculata* sampled in plot 1; T3) *Hippodamia convergens* sampled in plot 1; T4) *Harmonia axyridis* sampled in plot 2; T5) *Coleomegilla maculata* sampled in plot 2; T6) *Hippodamia convergens* sampled in plot 2. Pooled samples consisted of 2 µl of sample DNA plus enough distilled water to bring the total pooled sample volume to 24 µl. Blanks were not pooled.

*Metabarcoding sample preparation*

A two-step PCR (Berry et al. 2011) was performed on the pooled samples with each of the eight primer-pairs (Table 3.1). For the first step PCR reaction, each pooled sample and blank was amplified with a reaction mixture of 2 µl of pooled DNA (water in the blanks) and the final concentrations of 10x PCR buffer [1.5 mM $MgCl_2$, 10 mM KCl, 8 mM $(NH_4)_2SO_4$, 10 mM Tris-HCl, pH9.0, 0.05% NP-40], 0.16 µl dNTP mix (25 µM each), 0.5 µM of each primer, 2 µg of Bovine Serum Albumin (BSA), and ChoiceTaq® DNA Polymerase 1.25 µl in a total volume of 20 µl. For each primer-pair, a non-template control (NTC) was run to check for DNA cross-contamination. Reactions were amplified in either an Eppendorf Mastercycler Gradient or an Eppendorf Mastercycler® Pro using a touchdown PCR program (Leray et al. 2013): 1 cycle of denaturation at 94°C step for 4 min, 15 cycles of three steps [denaturation at 94°C for 30 s, primer annealing at 65°C for 30 s (decreased 1°C per cycle), and extension at 72°C for 60 s], 20 cycles of three steps [denaturation at 94ºC for 30s, primer annealing at 50ºC for 30 s, and extension at 72ºC for 60 s], and no final elongation step. The presence of an amplicon band of the expected size for each primer-pair was evaluated by electrophoresis in 1x Tris-Borate-EDTA (TBE) buffer (Sambrook and Russell 2006) and 2% agarose gel pre-stained with 1x Biotium GelGreen® Nucleic Acid Gel Stain and visualized using a SmartBlue® Blue Light Transilluminator at 465 nm.

The second PCR reaction was performed in triplicate for each of the six primer-pairs for *cox1*, the 16S primer-pair, and the ITS primer-pairs using the same touchdown PCR program as above. The forward primers had a unique 6-nucleotide tag for each pooled sample (Coissac et al. 2012) and the reverse primers had the same tag (Table S3.2). The amplicons from the first PCR served as templates for the second PCR with similar conditions as the first PCR. Reagents were adjusted for the same concentrations in a final volume of 50 µl. As no amplification was observed in the blanks after the first PCR, they were not included in the second PCR. New NTCs were performed for each uniquely tagged primer-pair. Triplicate amplicons from the same pooled sample from each of the eight tagged primer-pairs were combined, checked for amplification by electrophoresis as described above, and purified using a Monarch PCR & DNA Cleanup

Kit (NEB) following the manufacturer protocol. The purified amplicons were quantified in duplicate with Qubit® 3.0 fluorometer (Thermo Fisher Scientific) using a Qubit dsDNA HS Assay Kit. Purified tagged amplicons from each sample and primer-pair were combined at equimolar concentrations of 140 x $10^{-6}$ nmoles in one library, which was based on the minimum amplicon concentration among pooled samples. The multiplex sample was completely dried in an Eppendorf Vacfuge® Concentrator Plus at 60ºC at 131x*g* and shipped to Genome Quebec Innovation Centre at McGill University (Canada) for construction of one NEB Ultra II library (480 bp insert size) and sequencing by Illumina HiSeq2500 Rapid Mode (250 bp, paired-end, 1/7 of a lane).

*Bioinformatic analysis*

Metabarcoding data were processed using scripts available in the Supporting Information (Appendix 1, Chapter 3 SI). First, Fastqc was run on the raw read files prior to demultiplexing and no reads were flagged as poor quality. Paired-end reads were aligned, merged and demultiplexed using OBITools v.1.01 (http://metabarcoding.org/obitools) (Boyer et al. 2016). Using the *illuminapairedend* command, pairs of reads were aligned and the resulting consensus sequences with a quality score < 40 were filtered out. Aligned paired-end reads were assigned to respective samples (from T1 to T6) with the *ngsfilter* command using exact matches for tags and up to two mismatches for primers. *Obiconvert* and *obisplit* were used to convert and split the fastq file into sample FASTA files. The sample FASTA files were processed using four workflows (Fig 3.2). Three of the workflows started with a wrapper script NAPcluster (https://github.com/tjcreedy/NAPtime) to generate OTUs. This script utilizes USEARCH (v.9.2) UPARSE-OTU greedy algorithm with a 3% cluster parameter value and 3 bp variation around target sequence length, removing singletons (Edgar and Bateman 2010). For each OTU, the majority consensus sequence was extracted to assign taxonomy. Taxonomic assignment of sequences was achieved via the same OTU-forming wrapper script which implements a local BLAST to a reference database either (workflow 1) containing all available local NCBI nucleotide data (updated 6/17/18) or (workflows 2-4) a combined NCBI-BOLD database of Arthropoda nucleotide data (updated 8/20/19) based on the database building protocols of Macher et al. (2017). Hits from the NCBI-

BOLD BLAST were sorted into either (workflow 2) NCBI-BOLD loose (all hits) or (workflow 3) NCBI-BOLD strict (100% coverage 95% identity) hit pools. In a separate pipeline (workflow 4), unclustered, demultiplexed reads were matched to the NCBI-BOLD database with a local BLAST and assigned taxonomically with the least common ancestor algorithm (LCA) implemented in MEGAN Community Edition (v.6.16.4) with default settings except MinSupport ≥ 2 reads (Huson et al. 2007). For clustered workflows the top 25 BLAST hits were inspected to determine read identity. In total, four bioinformatic workflows were used: 1) Local NCBI, 2) NCBI- BOLD loose, 3) NCBI-BOLD strict, and 4) MEGAN (Fig. 3.2). Reads or OTUs used for taxonomic assignment from the samples in the four bioinformatic workflows were mapped back to a reference alignment in Geneious created during the aforementioned metabarcoding primer-design process. Taxon queries with a >98% identity with the reference alignment were selected for verification using Melting Curve Analysis (MCA).

*Prey detection validation by Melting Curve Analysis*

A list of prey taxa was compiled from metabarcoding results from the four bioinformatic workflows as well as from a field survey and knowledge of common coccinellid prey in the locale at the time of the collection (Table 3.3). Species-specific primers were then designed by downloading from BOLD and NCBI databases all available *cox1* sequences for each candidate prey taxon and aligning Folmer regions in AlleleID (v7.85) (Apte and Singh 2007). Species-specific primers were initially designed in AlleleID (default parameters set for SYBR® Green qPCR Assays) to avoid cross-amplification of the candidate prey taxa DNA within the same order. Next, Primer-BLAST (Ye et al. 2012) with default parameters was used to search the 'Arthropoda (taxid:6655)' subset of the nucleotide and protein non-redundant (nr) database to test in silico the specificity of the species-specific primers.

To test the efficacy and specificity of the designed species-specific primers (Table 3.3), DNA from available potential prey species was extracted (CTAB method, Appendix 1 Chapter 3) to work as a positive control. Prior to this, specimens were washed in a 2.5% bleach solution on a platform set to gentle agitation at 150 rpm for 40 min to remove external DNA (Greenstone et al. 2012), followed by standard DNA extractions. For

qPCR, reactions contained 6.5 µl Maxima SYBR Green/ROX qPCR Master Mix, 0.3 µM primers, 1 ng DNA, and dd$H_2O$ to complete 13 µl per reaction. The reactions were amplified in a LightCycler® 480 Instrument II, under either a two-step or three-step program, ending in a prolonged 'melting' cycle to denature amplicons and determine melting temperature (Table S3.3, Berry and Sarre 2007). Melting curve analysis (MCA) can reliably differentiate between closely related species given that even single nucleotide polymorphisms between amplicons can result in distinct melting temperature peaks (Taberlet et al. 2018). Primer-pairs were tested against prey taxa within the same family and were considered specific when they did not amplify the other potential prey species. Melting temperature (Tm) was determined for each available potential prey species (Figure S3.2).

MCA was conducted on the original purified pooled predator DNA (T1 to T6) in triplicate to verify candidate prey taxa detected in the metabarcoding analysis (Berry and Sarre 2007). Control qPCR reactions with prey species DNA were included when available. The threshold for prey detection by MCA was at least two of the three technical replicates displaying a melting curve peak of ≥ 0.5 -Δ(Mean Fluorescence Intensity/Time) above background within 1ºC of the control prey DNA. Melt curves were constructed in the MBmca package in R (R Core Team 2018) with the *mcaPeaks* function (Rödiger et al. 2013) (Fig S3.3).

*Data Analysis*

Prey taxa were deemed true positives (TP) when detected by both metabarcoding and MCA. False positives (FP) were prey taxa detected by metabarcoding but not MCA, and false negatives (FN) were prey taxa detected by MCA but not metabarcoding. For each metabarcoding primer-pair and bioinformatic method we compiled scores of TP, FP, and FN for each pooled predator sample. From the rates of TP, FP, and FN for each metabarcoding primer-pair and bioinformatic method combination, three additional metrics of prey detection were calculated: total positives (P=TP+FP), sensitivity (TP/TP+FN), and precision (TP/P).

Generalized linear mixed models (glmm) were fit with the *glmer* function in the lme4 package in R with pooled DNA sample as a random factor (Bates et al. 2015). To

assess whether primers designed specifically for diet-analysis of insectivorous arthropods in agroecosystems were more sensitive in detecting a broader range of prey taxa, the Poisson responses of P, TP, FP and the binomial response of sensitivity were modeled as a function of metabarcoding primer, bioinformatic method and their interaction. To test if length of amplicon overlap between pairs of *cox1* primers detected more similar prey, a Jaccard similarity coefficient among prey detected by different primers was calculated with the *vegdist* function in the vegan package in R. Separate coefficients were calculated for P and TP prey subsets. Logistic regression was used to model the Jaccard similarity between two primer-pairs as a factor of the overlap length (bp) between amplicons for both P and TP prey. Relative reads assigned to a given primer pair was modeled as binomial response (assigned, not assigned) of amplicon length, sample and their interaction to determine if longer amplicons returned fewer reads from the shared Illumina lane. FP were modeled as a factor of amplicon length, sample, and their interaction to investigate if longer amplicons reduced FP hits. To determine whether read clustering would decrease false-positive detections, FP was modeled as a function of bioinformatic clustering (Y/N), primer and their interaction. To investigate whether incorporating the MCA analysis was a necessary validation step, TP was modeled as a function of the HTS relative read abundance, primer, bioinformatic method and their interactions. As none of the three-way interactions or any term including method was significant in this model, these terms were dropped from the model.

For all models, the ratio of Pearson's residuals to residual d.f. was inspected to check for overdispersion, and factor significance was tested with a Type II ANOVA, extracted mean separation between factor levels with a Bonferroni correction, and visualized significant factors with *ggplot* (Wickham 2011). To compare coccinellid predator diet results generated by metabarcoding alone (P) or with the MCA verification step (TP) separate food webs were constructed to calculate species richness and directed connectance (links/species$^2$) found between the approaches. All statistical analysis was performed in R (v3.5.1) (R Core Team 2018).

**Results**

Overall, fifteen potential prey taxa were detected by metabarcoding combining all seven primer-pairs used to detect arthropods (ArF10/R3, BF1/R1, BF1/agroR2, agroF1/R1, Unimini, ZBJ and 16S) and all the bioinformatic pipelines (clustering reads with Local NCBI, NCBI- BOLD loose and strict; and unclustered reads by MEGAN): *Anaphothrips obscurus*, *Aphidius ervi*, *Coleomegilla maculata*, *Dinocampus coccinellae*, *Drepanaphis acerifoliae*, *Entomobrya quadrilineata*, *Harmonia axyridis*, *Hippodamia convergens*, *Hysteroneura setariae*, *Macrosiphum euphorbiae*, *Orius insidiosus*, *Pemphigus populitranversus*, *Pieris rapae* and *Sitobion avenae*. MCA confirmed only seven prey species: *A. ervi*, *D. coccinellae*, *H. axyridis*, *H. setariae*, *P. populitranversus*, *P. rapae* and *S. avenae*, , despite MCA primers amplifying significantly shorter prey fragments than metabarcoding primers (t=2.17, df=17, p=0.043). Two prey species (*Rhopalosiphum maidis* and *Trichoplusia ni*) not found in the metabarcoding results were detected as FN by MCA. Additionally, the coccinellid *Cycloneda munda* was detected as FN prey item in one predator (*Harmonia axyridis* in plot1) and a FP in two other samples (*Harmonia axyridis* and *Hippodamia convergens* in plot2).The ITS primer was used to detect host plants, however no plants were detected to species and no plants were tested by MCA.

*Primer effect on prey detection*

The primers designed specifically for entomophagous arthropod diet analysis did not significantly improve metabarcoding efficacy (Fig. 3.3, Table S3.4). While all seven arthropod primers detected the coccinellid predator *Harmonia axyridis* as an intraguild prey and six primers detected the coccinellid parasitoid *Dinocampus coccinellae,* no single primer detected >55% of the taxa verified by MCA (Table 3.4). *Harmonia axyridis* and *D. coccinellae* were the prey/parasitoid items detected with the most abundant reads. On average 23.32% (SE =0.68%) and 41.62% (SE=0.71%) of reads were assigned to *H. axyridis* and *D. coccinellae* respectively across primer pairs and bioinformatic methods. Among the six *cox1* primer-pairs, there was no relationship between similarity of prey detected and amplicon overlap length along the Folmer region ($\chi^2$= 1.095, $p$ = 0.2954; Fig. 3.1). All primers designed for mixed arthropod DNA (agroF1/R1, BF1/R1, BF1/agroR2, ZBJ) had similar rates of TP detection, with an average of 1.08-1.25

(SE=0.10-0.11) taxa detected per predator (Fig. 3.3a, Table S3.4). Both *cox1* primers designed for narrower (ArF10/R3, Hemipteran targets) or broader applications (Unimini, eukaryote targets) detected < 0.9 prey taxa per predator on average. True positives and FP were overall similar among all the *cox1* primers with only the16S primer detecting significantly fewer FP (0.95, SE=0.16) than the ZBJ primer-pair (3.42, SE=0.27) (Fig. 3.3a, Table S3.4). On the other hand, the 16S primer only detected one TP prey (*H. axyridis*) (Table 3.4). ZBJ primer had the highest number of FP prey species detected ($n$=10), although not significantly different from the other *cox1* primers (range = 4-10 FP taxa). False positives declined with increasing metabarcoding amplicon length, as predicted ($\chi^2$=8.77, $p$=0.003). However, the relative number of reads assigned to a primer pair declined significantly with length ($\chi^2$=4.75, $p$=0.029). There were no significant differences in the precision of the primers, indicating similar proportions (0.28, SE =0.01) of FP among prey detected by each primer-pair (Fig 3.3a, Table S3.4). *Cox1* primers tended to be more sensitive, detecting more positive hits overall than the 16S primer-pair, but not significantly so (Fig. 3.3b, Table S3.4). Only the agroF1/R1 and ZBJ primers were significantly more sensitive (both 0.53, SE=0.05) than the 16S primer (0.09, SE=0.04) (Fig. 3.3b). The FP taxa detected by metabarcoding and associated read counts using the seven arthropod metabarcoding primers are shown in Table S3.5.

*Bioinformatic pipeline effect on prey detection*

The clustered pipelines detected the same six TP prey taxa (*H. axyridis*, *P. populitranversus, S. avenae, A. ervi, D. coccinellae,* and *P. rapae*). MEGAN, using a least-common ancestor algorithm to parse unclustered read hits detected four TP prey taxa, of which one (*H. setariae*) was unique, but failed to detect *A. ervi, P. populitranversus* and *S. avenae*, which were detected by the other pipelines (Table 3.5). All taxa detected across bioinformatic methods were represented in both BOLD and NCBI databases. Clustering reads into OTUs prior to taxonomic assignment by BLAST produced a significantly higher frequency of FP compared to the MEGAN method (Tables S3.4, S3.6). On average, clustered methods detected 2.4 (SE=0.11) FP per sample whereas the unclustered MEGAN method detected 1.81 (SE=0.13) FP per sample. Clustering had no effect on TP detections ($\chi^2$=0.57, $p$=0.45). Incorporating *cox1*

sequences from the BOLD Systems database into a BLAST reference database did not increase the number of prey species detected as measured by overall positives or true positives compared to the smaller database consisting of only NCBI *cox1* sequences ($\chi^2$=6.96, $p$=0.07; $\chi^2$=2.61, $p$=0.45, respectively). On average methods using the BOLD-NCBI detected 3.17 (SE=0.01) P and 0.92 (SE=0.01) TP, whereas the method using NCBI database alone detected 3.00 (SE=0.04) P and 0.73 (SE=0.01) TP hits.

*Relative read abundance related to true prey taxa detected*

The likelihood that a given taxonomic hit was a TP verified by MCA increased with relative read abundance (RRA) for two of the seven metabarcoding primers, ArF10/R3 and ZBJ (Wald $\chi^2$=4.19, p=0.041; Wald $\chi^2$=5.749, p=0.017) (Tables S3.4, S3.7). Overall, individual read counts for TP and FP taxa demonstrated the low reliability of RRA as a threshold criterion to accept or reject a taxonomic hit. We found no relationship between RRA of taxa in metabarcoding and the relative initial template concentration estimated by in MCA ($r^2$= 0.04, $p$=0.73; Fig. S3.4). True-positive prey verification by MCA occurred for taxa with as few as 1-2 reads (*e.g.*, *Aphidius ervi* and *Pieris rapae* by the agroF1/R1 primer pair, Table 3.4). Conversely, several intraguild coccinellid prey were false positives despite having >1,000 reads, including all detections of *Coleomegilla maculata* and *Hippodamia convergens* as intraguild prey items (Tables S3.6, S3.7).

*Relevance of checking metabarcoding results in food web studies*

Removing false-positive prey detections from metabarcoding food webs resulted in less speciose and connected food webs (Fig. 3.4). There were fewer FN than FP prey detected by MCA verification, therefore the food web estimated by metabarcoding overestimated species richness by 41.6% (metabarcode = 17, MCA = 12). Metabarcoding alone also inflated the level of directed connectance (links/species$^2$) (metabarcode = 0.142, MCA = 0.097) in the food web, with over double the number of links between species in metabarcoding alone than after MCA verification. Further, MCA verification allowed diet characterization of individual beetles, revealing that the proportional

estimates of interaction strength were on average 3.37 (SE=0.80) times higher when based on pooled samples than individual samples.

**Discussion**

Before metabarcoding can be reliably applied for diet studies in entomophagous arthropods, results must be clear, reliable and repeatable. This study documented errors, both FP and FN from metabarcoding of gut contents with all seven primer-pairs and bioinformatic workflows. Though many of the primers amplified overlapping regions of the *cox1* Folmer region, we found no relationship between relatedness of primers and ability to detect similar prey. The primers designed in this study were aimed to specifically detect arthropod prey most relevant to agroecosystems, yet the new primers did not improve prey detection over published primers. The analysis did not identify a dominant primer, capable of detecting all taxa detected by other primer pairs, indicating that multiple universal primers may be necessary to detect a full diet breadth. By employing a MCA verification step to metabarcoding taxonomic hits, we demonstrated that read abundances generated in metabarcoding were a poor predictor of TP prey detection and should be treated with caution. Creating an accurate food web from the coccinellid diet study suggests that combining metabarcoding and MCA analysis will generate less complex food webs than if metabarcoding data was used alone.

Metabarcoding has the potential to detect a broad diversity of taxa within a mixed DNA sample, yet the results indicate that common FP and FN errors may limit the reliability of metabarcoding results alone. False negative errors could originate from amplification biases during the PCR step, which are more readily identified in mixed bulk sample analysis, where morphospecies abundance and/or biomass can be compared to post-sequencing results (Elbrecht and Leese 2015, Taberlet et al. 2018). In metabarcoding dietary studies, mock communities (Galan et al. 2018, Jusino et al. 2019), and more commonly controlled feeding trials (Srivathsan et al. 2015, Macías-Hernández et al. 2018, Jusino et al. 2019, Thuo et al. 2019), have been used to detect primer biases and avoid FNs. However, neither approach can incorporate the full complex set of biological variables associated with gut content analysis (e.g., digestion) that can influence amplification bias in field collected samples (Alberdi et al. 2019).

Among the seven primers tested, none was able to recover the full set of TP prey species detected and all of them failed to detect FN prey identified by MCA. Given that previous work has shown that primer biases are largely attributable to primer-base mismatch (Jusino et al. 2019), we were surprised that incorporating high levels of degeneracies into the agro primers to accommodate primer-binding site mismatches among target prey, did not increase the number of prey detected over established primers. AgroF1/R1 was the only primer pair to detect thrips, but this interaction could not be verified by MCA. A key trade-off in selecting a metabarcoding primer is whether to prioritize taxonomic resolution (e.g., for a prey of interest) or taxonomic coverage (over all conceivable prey), and this approach to primer selection leaned towards the latter strategy. However, even the narrowly targeted primer-pair ArF10/R3 (designed for Hemipteran detection), did not reliably return hemipteran aphid prey in these samples. As suggested by previous works (De Barba et al. 2014, Gibson et al. 2014), these results support using multiple well-performing metabarcoding primers on a single sample to reduce FN errors. Potentially paring a conserved organelle genome barcode with a nuclear genome barcode could balance tradeoffs between FN and FP errors.

This study indicates that approaches relying upon read abundances to identify FP taxonomic hits are likely to come at the cost of increasing FN errors (Ficetola et al. 2016, Lahoz-Monfort et al. 2016). Bioinformatic workflows in this study took used liberal parameters to retain rare TP reads that likely would have been eliminated in pipelines that require reads to pass count or percentage thresholds (Corse et al. 2017). Valid, but rare prey can also be removed in workflows that require detection in multiple technical replicates or by multiple primer-pairs, despite subsampling procedures that could miss rare prey during metabarcoding library preparation (Leray and Knowlton 2017). These results showed that the detection of a prey taxon by multiple primers did not guarantee a TP hit, at least when considering primers targeting a close amplification site or a same genome region or genetic compartment (e.g., mitochondrion) as the ones used in this work. Statistical methods such as site occupancy modeling have been proposed to identify FPs, however these results do not meet the assumption of higher TP than FP detections (Ficetola et al. 2016, Lahoz-Monfort et al. 2016) (Fig 3.3). Read clustering into OTUs is another proposed solution to minimize FP from erroneous sequences, yet

the data show an increase of FP hits in the bioinformatic methods using OTUs over an unclustered approach. This result be attributable to the LCA algorithm used to process unclustered reads rather than inspecting top hits of OTU consensus sequences. In this study different primers (sometimes all of them) detected the same false positive prey across all bioinformatic workflows, indicating some level of taxonomic overdetermination during the data processing.

The use of a downstream method that can access the original DNA community composition in a sample (e.g., species diagnostic primers, validated by MCA) proved to be an important alternative taxonomic verification step when prey can be identified to species level. In these results, read counts were not only inappropriate to quantify diet components from gut-contents as indicated by Deagle et al. (2018), but also to assess which sequences were lingering false-positives. Read counts of false-positive coccinellid intraguild prey were five orders of magnitude higher than non-intraguild prey deemed true-positives (Table 3.4, S3.5). This could indicate an early amplification error resulting in misassignment of predator amplicons as a closely related coccinellid prey, or a degree of tag-jumping between samples, which can occur at several points in the metabarcoding workflow (Schnell et al. 2015). Misassignment of intraguild predators is particularly difficult to identify without a secondary verification step when closely related taxa are consumed. Some studies advocate for the use of predator-blocking oligonucleotides to diminish the occurrence of predator DNA (Shehzad et al. 2012, Masonick et al. 2019). However, Piñol et al. (2015) demonstrated that among arthropods in a mixed bulk sample, an oligonucleotide targeted at blocking one species (e.g., predator in gut content analysis) can significantly reduce amplification of closely related species (e.g. intraguild prey in gut content analysis). By using MCA to verify taxonomic prey hits we were able to retain all TP intraguild prey and efficiently remove FP coccinellid hits.

The approach of combining metabarcoding with MCA reduced the overall diversity and connectance of the food web, displaying species-specific differences in the propensity of these predators to consume herbivore and intraguild prey (Fig 3.4, Chapter 4). Neither method alone could have detected all of the prey in the coccinellid food web. The strength of metabarcoding in this system was to caste a broad taxonomic net to capture prey we would not have identified a priori. Two detected aphid species, *H.*

*setariae* and *P. populitranversus* are not commonly found in agricultural areas in the sampling region (Minnesota) and therefore would have been missed if we had only relied upon a priori knowledge of prey and taken a species-specific PCR approach. Alternatively, had we relied solely upon metabarcoding results, we would have overestimated both target-prey and intraguild prey consumption. The combined approach revealed that only one of the three coccinellid predators, *C. maculata* consumed herbivore *Brassica* pest prey and rarely consumed intraguild prey. With such knowledge, *Brassica* farmers could take actions to promote the conservation of specifically *C. maculata* rather than all entomophagous arthropods.

Metabarcoding is increasingly used in biosurveillance programs aimed at detecting pest and invasive species in natural and agroecosystems (Piper et al. 2019, Sousa et al. 2019, Westfall et al. 2020). Improving the application of metabarcoding to diet analysis by incorporating MCA verification will allow research to delve beyond questions of which arthropods are present in an ecosystem to the question of which arthropods are interacting. As FP and FN prey taxa detection is not simply addressed by the choice (or design) of metabarcoding primers and or bioinformatic pipeline, it seems reasonable to proceed with a downstream verification step for validation of metabarcoding analysis of gut contents from field collected predators (i.e., unknown history of predation), at least for most sensitive applications of metabarcoding (e.g., food web construction). In the context of invasive or pest management, integrating MCA analysis (or other downstream independent verification step) with metabarcoding could improve confidence in trophic relationships and improve understanding of how these relationships reorganize in response to environmental changes such as habitat management, climate change, or introduction of a non-native species.

**Table 3.1.** Primer pairs used for the metabarcoding dietary analysis of three coccinellid species (*Harmonia axyridis*, *Hippodamia convergens*, and *Coleomegilla maculata*) from plots of collards (*Brassica oleracea*). The following IUPAC notation indicates degenerate bases within a primer sequence: W(T or A), Y(T or C), R(G or A), K(G or T), D(not C), H(not G), and N or I(any base).

| PCR assay | Target Taxa | Gene | Primer-pair | Primer sequence (5'-3') | length (bp) | degeneracy | Reference |
|---|---|---|---|---|---|---|---|
| BF1/R1 | freshwater macroinvertebrates | *cox1* | BF1 | ACWGGWTGRACWGTNTAYCC | 257 | 1024 | Elbrecht & Leese 2017 |
| | | | BR1 | ARYATDGTRATDGCHCCDGC | | | |
| BF1/agroR2 | agricultural arthropods | *cox1* | BF1 | ACWGGWTGRACWGTNTAYCC | 246 | 4096 | Elbrecht & Leese 2017 |
| | | | agroR2 | WGCHCCDGCWARWACWGG | | | herein |
| agroF1/R1 | agricultural arthropods | *cox1* | agroF1 | GCHCCWGAYATRGCHTTYCCHCG | 119 | 1024 | herein |
| | | | agroR1 | ACWGTTCAWCCWGTHCCRWYHCC | | | |
| ArF10/R3 | Hemipterans | *cox1* | ArF10 | CCWGATATAKCITWYCCICG | 355 | 131072 | Gibson et al. 2014 |
| | | | ArR3 | GTRATWGCICCIGCTARWACWGG | | | |
| Uni-Mini | eukaryotes | *cox1* | Uni-Minibarcode_F1 | TCCACTAATCACAARGATATTGGTAC | 127 | 2 | Meusnier et al. 2008 |
| | | | Uni-Minibarcode_R1 | GAAAATCATAATGAAGGCATGAGC | | | |
| ZBJ | aerial arthropods (bat diet) | *cox1* | ZBJ-ArtF1c | AGATATTGGAACWTTATATTTTATTTTTGG | 157 | 8 | Zeale et al. 2010 |
| | | | ZBJ-ArtR2c | WACTAATCAATTWCCAAATCCTCC | | | |
| 16S | insects | *rrnL* | Ins16S_1short_F | TRRGACGAGAAGACCCTATA | ~216 | 4 | Clarke et al. 2014 |
| | | | Ins16S_1short_R | ACGCTGTTATCCCTAAGGTA | | | |
| ITS | angiosperms | ITS | ITS-u1 | GGAAGKARAAGTCGTAACAAGG | ~280 | 16 | Cheng et al. 2016 |
| | | | ITS-u2 | GCGTTCAAAGAYTCGATGRTTC | | | |

"F" and "R" refer to forward and reverse primers respectively

**Table 3.2.** Summary of taxa used as reference to design agronomic entomophagous arthropod primers. (See Table S3.1 for family-level read information).

| | | sequences | | |
|---|---|---|---|---|
| **Order** | **no. families** | **input** | **dereplicated** | **clustered** |
| Acari | 8 | 32213 | 15672 | 2587 |
| Araneae | 1 | 89819 | 41556 | 4345 |
| Blattodea | - | 7135 | 4456 | 1430 |
| Coleoptera | 38 | 250109 | 122511 | 28441 |
| Collembola | - | 123786 | 48557 | 5569 |
| Dermaptera | 2 | 1229 | 390 | 42 |
| Diptera | 29 | 1266620 | 507703 | 40571 |
| Hemiptera | 36 | 213478 | 89287 | 10556 |
| Hymenoptera | 27 | 360699 | 179940 | 28156 |
| Isoptera | - | 3226 | 1910 | 640 |
| Lepidoptera | 31 | 742823 | 307229 | 62427 |
| Mantodea | 1 | 998 | 586 | 236 |
| Neuroptera | 2 | 4757 | 2627 | 264 |
| Orthoptera | 3 | 19634 | 11491 | 2275 |
| Phasmatodea | 1 | 1010 | 618 | 146 |
| Psocoptera | - | 9012 | 5467 | 446 |
| Strepsiptera | - | 625 | 385 | 128 |
| Thysanoptera | 3 | 25347 | 9077 | 896 |

**Table 3.3.** Species-specific primers designed for validation of prey detection through Melting Curve Analysis. PCR program with annealing at 60ºC had extension at the same temperature (qPCR program details in Table S3). Source column indicates method of candidate prey taxa selection: observed during field survey taken the same day as predator collections; environment cited in the literature where taxa were known to occur in at the time of growing season (August, Minnesota); and metabarcode refers to taxa detected in the metabarcoding analysis. Primer name indicates the species to detect, main software used in the primer design, and if the primer was designed using the *cox1* Folmer region or the whole mitogenome (mtg).

| Order | Family | Species | Source | true+ | Name | Primer sequence (5'-3') | Length (bp) | Tm |
|---|---|---|---|---|---|---|---|---|
| Coleoptera | Chrysomelidae | *Phyllotreta striolata* | observed | Y | P.str_AlleleID_ cox1_F | CCTTATTTGTGTGAGCAGTAT | 88 | 60 |
| | | | | | P.str_AlleleID_ cox1_R | GTGAGCAGTATTGTGATAGC | | |
| | Coccinellidae | *Coleomegilla maculata* | metabarcode | Y | C.mac_AlleleID_cox1_F | AATATGAGCAGGAATAATTGGTA | 190 | 60 |
| | | | | | C.mac_AlleleID_cox1_R | TATTAAAGGGACAAGTCAGTTC | | |
| | | *Cycloneda munda* | metabarcode | Y | C.mun2_Geneious_ cox1_F | TAGGTGCAGGAACAGGGTGA | 76 | 60 |
| | | | | | C.mun2_Geneious_ cox1_R | AGATCAACAGAGGGTCCGCT | | |
| | | *Harmonia axyridis* | metabarcode | Y | H.axy_AlleleID_mtg_F | AGGATACTAAGAAGGACTAAGGT | 200 | 53/73 |
| | | | | | H.axy_AlleleID_mtg_R | TGATGGAAGATAAGGCAAGAAT | | |
| | | *Hippodamia convergens* | metabarcode | Y | H.con_AlleleID_cox1_F | CTGGAATCTCTTCTATCTTA | 179 | 53/73 |
| | | | | | H.con_AlleleID_cox1_R | ATCAGTTAATAATATAGTAATTGC | | |
| Collembola | Entomobryidae | *Entomobrya quadrilineata* | metabarcode | related sp. | E.quad_AlleleID_ cox1_F | CTGGCTAGTACCTCTAAT | 117 | 53/72 |
| | | | | | E.quad_AlleleID_ cox1_R | TCAACTAATCCACCTACT | | |
| Hemiptera | Aphididae | *Acyrthosiphon pisum* | environment | Y | A.pis_PrimerBlast_mtg_F1 | CACGAGCATATTTCACATCAGCA | 166 | 60 |
| | | | | | A.pis_PrimerBlast_mtg_R1 | ACACCTGTTAACCCACCGAT | | |
| | | *Aphis glycines* | environment | Y | A.gly_PrimerBlast_ cox1_F1 | AGTAGCCCATTTCCACTACG | 214 | 53/72 |
| | | | | | A.gly_PrimerBlast_ cox1_R1 | TGGATAGTCTGTATATTGGCGAGG | | |

| Order | Family | Species | Source | true+ | Name | Primer sequence (5'-3') | Length (bp) | Tm |
|---|---|---|---|---|---|---|---|---|
| | | *Brevicoryne brassicae* | observed | Y | B.bra_AlleleID_mtg_F2 | GCAGCATTTACTTTAACAATCA | 94 | 60 |
| | | | | | B.bra_AlleleID_mtg_R2 | GACAACGAATAGCCGAAAT | | |
| | | *Drepanaphis acerifoliae* | metabarcode | N | D.ace_AlleleID_ cox1_F | AGTATTAGCAGGAGCCATTAC | 109 | 60 |
| | | | | | D.ace_AlleleID_ cox1_R | TCAGAATAAGTGTTGGTATAGGAT | | |
| | | *Hysterneura setariae* | metabarcode | N | H.set_PrimerBlast_ cox1_F | TTTAAGAATCTTAATCCGATTAGAACTT | 145 | 60 |
| | | | | | H.set_PrimerBlast_ cox1_R | GTTTCCAAATCCTCCAATAACAATA | | |
| | | *Macrosiphum euphorbiae* | metabarcode | Y | M.eup_PrimerBlast_ cox1_F1 | AGCTGGTGCAATTACAATACTTTT | 79 | 60 |
| | | | | | M.eup_PrimerBlast_ cox1_R1 | AGGATCTCCTCCTCCTGCTG | | |
| | | *Myzus persicae* | observed | Y | M.per_PrimerBlast_ cox1_F1 | GGGAGGTGACCCAATCTTGT | 242 | 53/72 |
| | | | | | M.per_PrimerBlast_ cox1_R1 | AGTATGCTCGTGTATCCACATCT | | |
| | | *Pemphigus populitransversus* | metabarcode | N | P.pop_AlleleID_ cox1_F | ATAACTATACCTATTGTAATTGGA | 170 | 53/73 |
| | | | | | P.pop_AlleleID_ cox1_R | CCTGTTCCTGTACCATTA | | |
| | | *Rhopalosiphum maidis* | environment | Y | R.mai_AlleleID_mtg_F2 | GGTGGTTTCGGAAATTGATTA | 174 | 53/72 |
| | | | | | R.mai_AlleleID_mtg_R2 | AGATAATGGTGGATAAATTGTTCA | | |
| | | *Rhopalosiphum padi* | environment | N | R.pad_AlleleID_ cox1_F | TTTCTCTACATTTAGCAGGAAT | 197 | 53/72 |
| | | | | | R.pad_AlleleID_ cox1_R | ACGATCAGTAAGAAGTATAGTAAT | | |
| | | *Sitobion avenae* | metabarcode | Y | S.ave_PrimerBlast_ cox1_F2 | CCAGCAGGAGGAGGGGAT | 257 | 60 |
| | | | | | S.ave_PrimerBlast_ cox1_R2 | ATGCTCGTGTATCAACGTCT | | |
| Hymenoptera | Braconidae | *Aphidius ervi* | metabarcode | Y | A.erv_AlleleID_ cox1_F | TGGTGGATTTGGTAATTGAT | 196 | 53/73 |
| | | | | | A.erv_AlleleID_ cox1_R | ACCTCTATGTCCTAAAGTCA | | |

| Order | Family | Species | Source | true+ | Name | Primer sequence (5'-3') | Length (bp) | Tm |
|---|---|---|---|---|---|---|---|---|
| | | *Dinocampus coccinellinae* | metabarcode | Y | D.coc2_AlleleID_ cox1_F | TAATAGAGGGGCAGGGACGG | 75 | 53/72 |
| | | | | | D.coc2_AlleleID_ cox1_R | ACAGAAATACCCCCATGCCT | | |
| Lepidoptera | Noctuidae | *Trichoplusia ni* | field | Y | Tri.ni_AlleleID_ cox1_F | TGGAATAGTAGGAACATCATTA | 105 | 53/72 |
| | | | | | Tri.ni_AlleleID_ cox1_R | TGAGCGGTTACAATAGTATT | | |
| | Pieridae | *Pieris rapae* | metabarcode | Y | P.rap_AlleleID_ cox1_F | GCAGGAACAGGATGAACA | 79 | 60 |
| | | | | | P.rap_AlleleID_ cox1_R | AAATGGCTAAATCTACTGAAGAAC | | |
| | Plutellidae | *Plutella xylostella* | field | Y | P.xyl_AlleleID_ cox1_F | CTATTGTTATTGGAGGATTC | 199 | 53/72 |
| | | | | | P.xyl_AlleleID_ cox1_R | TGAGCGATATTTGAAGATA | | |
| Trombidiformes | Tetranychidae | *Tetranychus urticae* | observed | N | T.urt_AlleID_ cox1_F | TTTCAGGACTTATAGGGACTTCAA | 113 | 60 |
| | | | | | T.urt_AlleID_ cox1_R | AGCGTGCGTTGTAACTATTG | | |

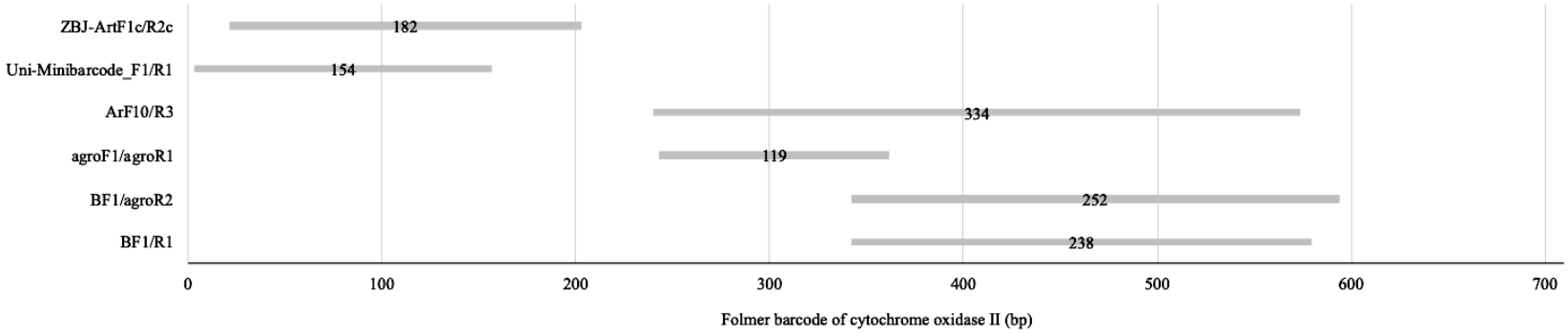
**Table 3.4.** Number of reads assigned to a **true-positive** prey taxon detected by each metabarcoding primer-pair and confirmed by MCA averaged across bioinformatic methods and coccinellid predators (*n*=6) detecting a given taxon. Numbers in parentheses indicated standard deviation.

| Order | Family | Species | 16S | Unimini | ArF10/R3 | BF1/R1 | BF1/agroR2 | agroF1/R1 | ZBJ |
|---|---|---|---|---|---|---|---|---|---|
| Coleoptera | Coccinellidae | *Harmonia axyridis* | 15.25 (0.43) | 3290.50 (464.30) | 53.50 (19.92) | 191.75 (54.13) | 568.25 (60.30) | 3094.50 (1029.02) | 2078.33 (1032.79) |
| Hemiptera | Aphididae | *Hysteroneura setariae* | - | - | - | - | - | - | 3.00 (0) |
| | | *Pemphigus populitranversus* | - | - | - | - | - | - | 47.00 (0) |
| | | *Sitobion avenae* | - | - | - | 4.00 (0) | 12.00 (0) | - | - |
| Hymenoptera | Braconidae | *Aphidius ervi* | - | - | - | - | - | 1.00 (0) | - |
| | | *Dinocampus coccinellae* | - | 7.33 (3.77) | 734.50 (19.92) | 29.33 (9.46) | 234.33 (51.85) | 10447.25 (2851.77) | 230799.75 (194562.97) |
| Lepidoptera | Pieridae | *Pieris rapae* | - | - | - | 8.00 (0) | 19.00 (0) | 2.00 (0) | 149.00 (168.29) |
| | | All Taxa | 15.25 (0.43) | 3296.00 (466.00) | 788.00 (00.0) | 222.75 (64.49) | 767.25 (95.83) | 13544.00 (3878.44) | 232518.75 (194679.74) |

**Table 3.5.** Number of reads assigned to a **true-positive** prey taxon for each bioinformatics pipeline and confirmed by MCA, averaged across primer pairs and coccinellid predators (n=6) detecting the given taxon. Numbers in parentheses indicate standard deviation.

| Order | Family | Species | MEGAN | local-NCBI-OTUblast | BOLD-NCBI loose | BOLD-NCBI strict |
|---|---|---|---|---|---|---|
| Coleoptera | Coccinellidae | *Harmonia axyridis* | 414.86 (850.64) | 1454.14 (1638.23) | 1456.29 (1641.25) | 1271.71 (1446.10) |
| Hemiptera | Aphididae | *Hysteroneura setariae* | 3.00 (0) | - | - | - |
| | | *Pemphigus populitranversus* | - | 47.00 (0) | 47.00 (0) | 47.00 (0) |
| | | *Sitobion avenae* | - | 8.00 (4) | 8.00 (4) | 8.00 (4) |
| Hymenoptera | Braconidae | *Aphidius ervi* | - | 1.00 (0) | 1.00 (0) | 1.00 (0) |
| | | *Dinocampus coccinellae* | 12422.33 (27444.670) | 4258.00 (5481.54) | 9268.17 (15436.05) | 70978.50 (157697.25) |
| Lepidoptera | Pieridae | *Pieris rapae* | 387.00 (0) | 9.67 (7.04) | 8.00 (6.75) | 8.00 (6.75) |
| | | All Taxa | 11118.29 (25759.17) | 3292.29 (5250.31) | 62124.14 (148087.25) | 9414.14 (15439.49) |

**Fig. 3.1.** Amplicon alignment by primer-pair to the *cox1* Folmer region. Table 3.1 gives exact primer sequences and references.



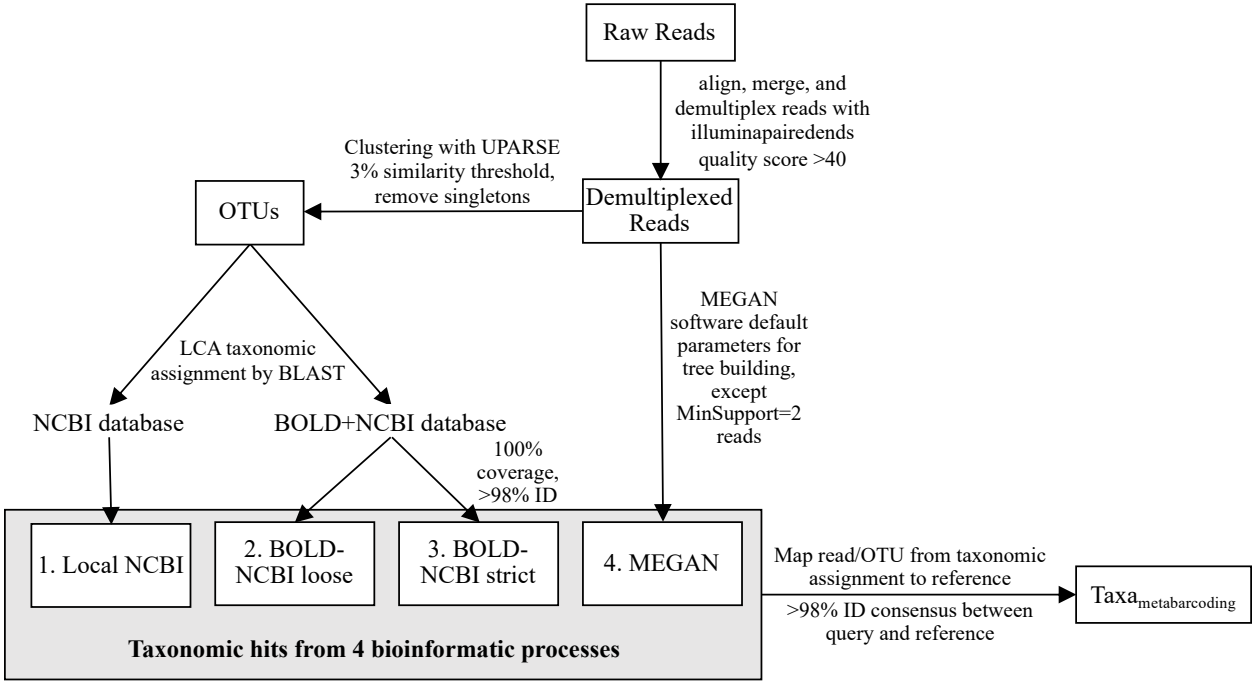



**Fig. 3.2.** Workflow for the four bioinformatic processes used to determine taxonomic assignment of metabarcoded reads from coccinellid predator guts.

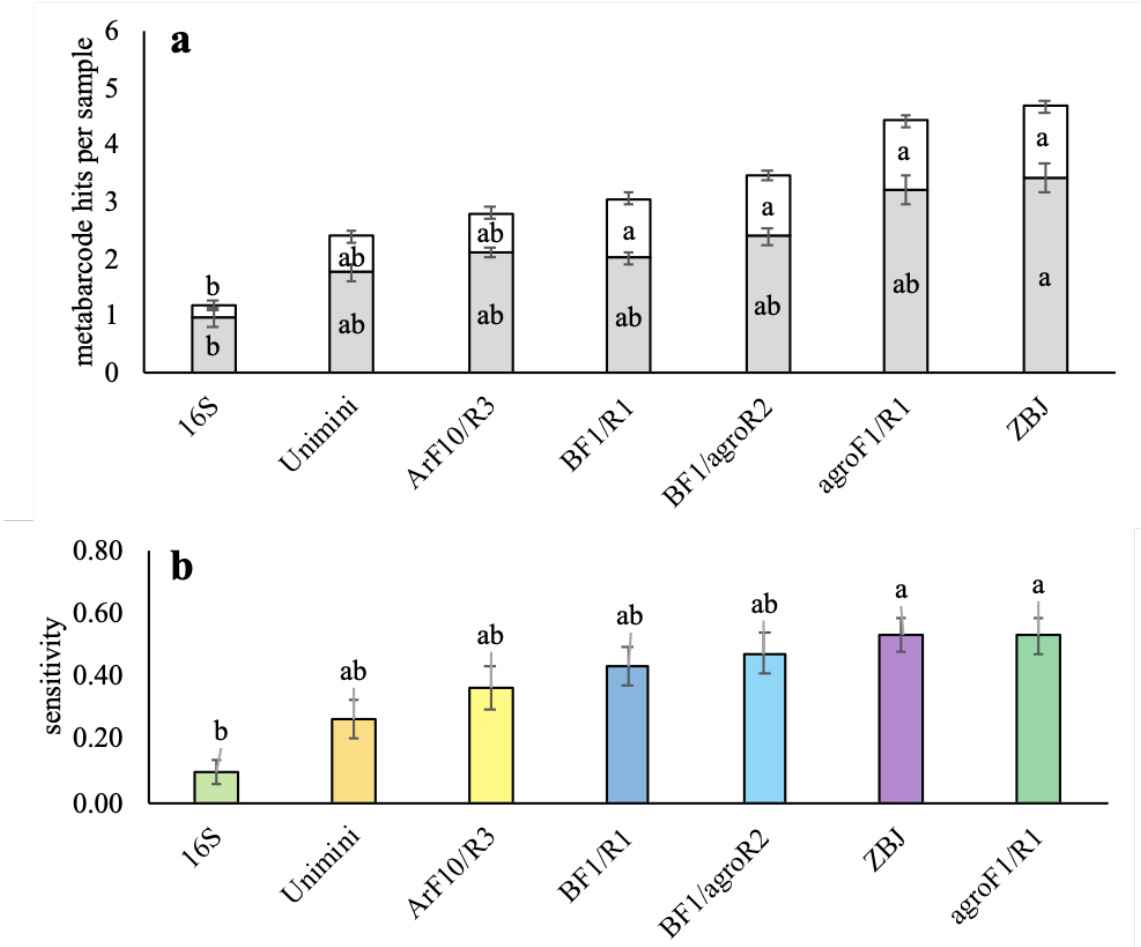



**Fig. 3.3. a)** Mean count of false positives (gray) and true positives (white) hits identified by metabarcoding primer-pairs. **b)** Mean sensitivity (true positives/total positives) of each primer pair. Error bars indicate SE. Letters indicate Bonferroni corrected mean separation. Means with the same letters do not differ significantly ($\alpha = 0.05$).

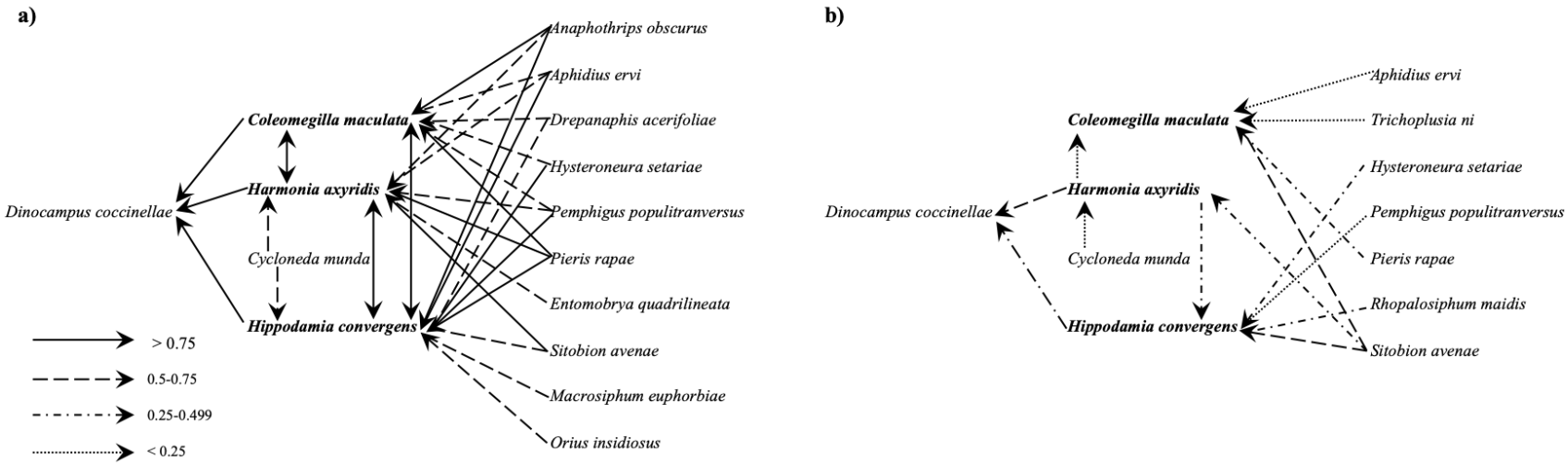



**Fig 3.4.** Food web results of gut content analysis of three coccinellid predator species (bolded) by **a)** metabarcoding alone or **b)** with a downstream independent verification method (melting curve analysis). Arrow pattern indicates relative proportion of predator individuals with a given prey detected (true detection melting curve analysis) as indicated by the scale in the lower left corner of panel **a)**. The hierarchy of the trophic levels is represented from right to left. Arrow orientation indicates direction of predation.

## Chapter 4: Coccinellid diet analysis reveals herbivore and intraguild prey consumption in a *Brassica* agroecosystem.


### Summary

Accurately identifying the diet of a predator is a crucial part of understanding its role in an ecosystem and capacity to control a resident prey population. The consumption of herbivores by coccinellids contributes significantly to pest control in many agroecosystems, yet competition over shared prey and consumption of intraguild prey can limit effectiveness. Efforts to determine coccinellid diets have historically focused on aphid species of economic importance, however DNA sequencing advances now allow for detection of a broader spectrum of prey. In this study we utilize both taxonomically broad metabarcoding and narrow melting curve analysis to characterize the adult diets of three coccinellid species (*Coleomegilla maculata, Harmonia axyridis,* and *Hippodamia convergens*) in a *Brassica* agroecosystem. We explore how the consumption of different prey types (herbivore, intraguild, resident, non-resident) and the influence of diet breadth on consumption of prey types varies among coccinellid species. These results show that all coccinellid species consumed the aphid *Sitobion avenae* but that no other herbivore species were shared prey. Only *C. maculata* consumed herbivore prey likely to damage the focal *Brassica* crop. All three species consumed intraguild prey frequently. Yet, *C. maculata* was less likely than the other two species to consume coccinellid prey and a broader diet increased the likelihood of *C. maculata* consuming herbivore-*Brassica* pests. *H. convergens* increased consumption of intraguild coccinellid prey with diet breadth. These findings indicate that comprehensive diet breadth analysis of arthropod predators under field conditions could improve understanding of which predator assemblages are likely to provide strong herbivore population control in agroecosystems.


### Introduction

Understanding the components of the diet of a predator is crucial to understanding its function in an ecosystem. While it is relatively simple to survey and quantify prey resources available for a predator, it is much more difficult to establish what a predator actually consumed under field conditions (Furlong 2015). A predator species that reliably

consumes a prey taxon in controlled laboratory experiments may not consume the same taxon in the field depending upon how it responds to habitat complexity, the presence of alternative preferred prey, or competition for shared prey with other predator taxa (Sloggett and Majerus 2000, Finke and Denno 2002, Jackson et al. 2017). Verifying diet breadth in the field may be particularly significant when attempting to determine whether a given predator species can adequately control a herbivore prey population alone or in the presence of other predator taxa.

Coccinellid beetles are beneficial insects in many agroecosystems where they consume herbivore prey taxa of economic concern. Aphids (Aphididae) are common herbivores of crops and are preferred prey of coccinellids in the subfamily Coccinellinae, though these predators will also consume other small-bodied arthropod prey opportunistically (Hodek and Evans 2012). Because coccinellid acceptance of prey often depends on prey size, many coccinellids also consume younger life stages of other predatory species (Roger et al. 2000, Rondoni et al. 2012). Such instances of intraguild predation, in which one predator species consumes another as part of its dietary niche, can disrupt the capacity of a predator community to regulate a resident herbivore population, and therefore merit equal consideration as herbivore predation when attempting to understand the trophic ecology of coccinellid species (Rosenheim 1998, Weber and Lundgren 2009).

Modern molecular gut content analysis has provided the opportunity to characterize the consumption of herbivores and intraguild prey by coccinellids. Metabarcoding, whereby universal primers amplify a barcode of high interspecific variability to identify taxa has been successfully used to study bat, fish, and leopard diets among other predatory taxa (Shehzad et al. 2012, Aizpurua et al. 2018, Siegenthaler et al. 2019). When employed to study coccinellid diets, metabarcoding could cast a broad taxonomic net and capture consumption of previously under-detected or novel prey. Additionally, combining a broad taxonomic molecular tool with efficient species-specific assays could verify prey detected by metabarcoding and identify any prey missed due to biases in metabarcoding (Berry and Sarre 2007). Paired together, these two tools can elucidate fuller and more reliable food webs than either tool alone.

In this study we used a novel combination of metabarcoding and species-specific molecular diet analyses to characterize the diet breadth of three coccinellid species (*Coleomegilla maculata, Harmonia axyridis,* and *Hippodamia convergens*) in a *Brassica* agroecosystem. By employing a combination of broad taxonomic metabarcoding and narrow species-specific melting curve analysis of coccinellid gut contents we detected both resident and non-resident prey and explored individual and species variation in prey consumption.

## Methods

### *Coccinellid collection, metabarcoding, and pooled melting curve analysis*

Methods for coccinellid collection, gut content DNA extraction, metabarcoding workflows, and validation of pooled prey detection by melting curve analysis are described in detail in the Materials and Methods section of Chapter 3 of this work.

In brief, coccinellid predators were collected on August 8$^{th}$, 2017 from two research collard plots (*Brassica oleracea*) separated by a 5 m tilled strip. Ten individuals of three species of coccinellids (*Coleomegilla maculata, Harmonia axyridis,* and *Hippodamia convergens*) were each collected from both collard plots. DNA was extracted from whole coccinellids individually with Monarch PCR & DNA Cleanup Kits, and then pooled by species and plot to create six pooled coccinellid samples for metabarcoding. Eight primer-pairs were used to extensively barcode the six pooled samples in order to detect a broad array of prey taxa in a two-step PCR protocol for sample tagging (Berry et al. 2011). All 48 purified and uniquely tagged amplicons for each primer pair and sample were combined at equimolar concentrations into one library for sequencing. The Genome Quebec Innovation Centre at McGill University constructed a NEB Ultra II library (480 bp insert size) and sequenced the library on an Illumina HiSeq2500 PE in rapid mode (250bp, 1/7$^{th}$ of a lane). Post sequencing, raw reads were quality controlled with Fastqc, then aligned, merged, and demultiplexed with an OBITools pipeline (v.1.01, http://metabarcoding.org/obitools) (Boyer et al., 2016). With the demultiplexed paired-end reads, four bioinformatic workflows were followed to generate taxonomic hits for each combination of primer-pair and sample (Fig. 3.2). These

four bioinformatic workflows generated a list of all taxonomic hits for each pooled sample. All bioinformatic scripts available in Appendix 1.

Taxonomic hits from metabarcoding for each pooled sample were verified with an independent qPCR melting curve analysis (MCA) on the original pooled DNA sample (Ficetola et al. 2016, Alberdi et al. 2018). A list of test prey species was compiled from all taxa detected to species by metabarcoding, prey observed in the plot at time of predator collection, and prey known to be common in the area at time of collection. No plants were detected to species level and therefore only potential arthropod prey species were validated. Species-specific primers for each taxon on the list were designed in Allele ID, Geneious, or PrimerBlast software (Table 3.3). Primers were vetted by testing against DNA isolated from control field collected specimens (Fig. S3.2). After verifying efficacy of qPCR primers against control prey DNA, the primers were tested against pooled predator DNA samples in triplicate (Fig. S3.3). Prey was deemed detected in a pooled sample when at least 2 of the 3 technical triplicate reactions displayed a melting curve peak of $\geq 0.5$ -$\Delta$(Mean Fluorescence Intensity/Time) within 1ºC of the control prey DNA. All MCA peaks were analyzed in the MBmca package in R (R Core Team 2018) with the *mcaPeaks* function (Rödiger et al. 2013).

*Individual coccinellid diet characterization*

If a prey species was detected in the pooled coccinellid DNA sample by MCA, that taxon was then tested for in all 10 individual coccinellid DNA samples that comprised the pooled sample. MCA was performed on individual coccinellid DNA with the same protocol as the pooled DNA. Detection was determined by the same criteria as set for the pooled sample. Only prey items verified by MCA detection were recorded as a consumed taxon for each coccinellid individual. Individual coccinellid DNA samples were deemed poor quality and removed from the analysis if neither prey nor predator DNA was detected by MCA.

*Host plant and prey assessment*

Surveys of the arthropod and plant community were conducted at the time of coccinellid collection to characterize the prey and prey resources present (Table 4.1, 4.2).

Two perpendicular point-intercept transects were used to characterize the weed plant taxa present in each plot. Plants were identified to genus and later cross-referenced with host plants of prey detected by metabarcoding and MCA to determine prey residency (Table 4.3). To characterize the prey community in each plot, twelve randomly selected collard plants and the plants in a 0.5 m radius around them were visually surveyed to census arthropod taxa present. Arthropods were identified to family and species where possible (Table 4.2).

*Data Analysis*

A quantified food web was created from results of individual coccinellid diets, combining results from both sampling plots. Food web species richness and connectance were calculated and prey species were categorized into prey types. Prey were organized into trophic guilds either as herbivore or natural enemy, and within those guilds into types of prey (lepidopteran, aphid, parasitoid, coccinellid) (Table 4.3) Prey species were categorized as non-resident prey if hosts did not occur within the plot, else they were labeled resident (Table 4.3). Intraguild prey species *Cycloneda munda* was deemed a resident prey based on the presence of coccinellid eggs in the plot and observations of *C. munda* adults earlier in the growing season (Table 4.2). The subcategories of herbivore-*Brassica* pest and natural enemy–coccinellid were delineated on the basis of economic impact to host plant and taxonomic family respectively. Counts and percent of individuals consuming each prey category and were tabulated by species (Table 4.4).

Consumption of each type of prey was modeled as a factor of predator species to test whether coccinellid predator species varied in propensity to consume different types of prey. As individual coccinellids only consumed one intraguild (natural enemy) taxon each, consumption of intraguild and intraguild-coccinellid prey was modeled as a binomial response. All other prey types were modeled as Poisson responses. Proportion of diet consisting of each prey type was also modeled as a binomial response of predator species, excluding individuals which consumed neither resident nor non-resident prey (Table 4.4). Differences in diet breadth among coccinellid species was tested by modeling the Poisson response of prey richness as a factor of coccinellid species.

To test whether the effect of diet breadth (prey richness) on prey consumption within a prey category varied by coccinellid species, logistic models were constructed with the binomial response of consumption (yes, no) as a factor of prey richness, coccinellid species, and their interaction. Overdispersion was tested for in all models by examining the Pearson's $\chi$ test statistic. Upon finding no overdispersion in models, the Wald $\chi^2$ test statistic was calculated to test for model significance and maximum likelihood estimates, their standard errors, marginal Wald $\chi^2$ scores, and their *p* values were extracted to aid in model interpretation.

**Results**

Prey were detected in 64% of coccinellid individuals (Table 4.4). The number of individuals without detected prey varied by coccinellid species, with more than two times the number of *H. axyridis* individuals lacking detected prey than the other two species (Table 4.4). The food web constructed from detected prey in coccinellids from both plots had a connectance value of 0.218, with a species richness of 11 and 12 links among species (Fig. 4.1). Number of prey species detected varied significantly by species (Table 4.5). The number of prey species detected in *H convergens* was significantly greater (mean=1.75, SE = 0.32) than in *H. axyridis* (mean = 0.53, SE = 0.18), but not significantly greater than in *C. maculata* (mean=1.3, SE = 0.21) (Table 4.4).

All except two coccinellid individuals which consumed prey, consumed herbivore prey, however the number of herbivore species consumed varied significantly among coccinellid species (Table 4.5). Both *C. maculata* and *H. convergens* individuals consumed significantly more herbivore species than *H. axyridis* individuals, which only consumed *Sitobion avenae* (Table 4.4, Fig. 4.1). *S. avenae* was the only prey species consumed by all coccinellid species (Fig. 4.1). *C. maculata* was the only predator species to consume herbivore-*Brassica* pests, two lepidopteran species, *Trichoplusia ni* and *Pieris rapae* (Fig. 4.1). *H. convergens* consumed three other species of aphid prey, *Hysteroneura setariae, Rhopalosiphum maidis,* and *Pemphigus populitransversus*, in addition to *S. avenae*.

Intraguild, natural enemy prey consumption was less common than herbivore prey consumption, yet 24% of coccinellid individuals and 37% of individuals which consumed

prey, consumed intraguild prey. Three species of intraguild prey were detected (Fig. 4.1). Both *C. maculata* and *H. convergens* consumed the third focal predator species *H. axyridis*. *H. axyridis* predators consumed a fourth species of coccinellid, *C. munda* and *C. maculata* consumed an aphid parasitoid, *Aphidius ervi* (Fig. 4.1). Individual predators consumed only one type of intraguild prey each. Neither the consumption of intraguild prey nor the proportion of intraguild prey in the diet of predators varied significantly among species (Table 4.4., Fig. 4.2). However, the consumption of coccinellid intraguild prey and the proportion of coccinellid prey in the diet of predators varied by species, with *C. maculata* least likely to consume another coccinellid species, and the diets of both *C. maculata* and *H. convergens* consisting of lower proportions of intraguild prey than *H. axyridis* (Table 4.4, Fig. 4.2).

Two detected aphid species, *H. setariae* and *R. maidis* were not observed in sampling plots and were unlikely to have been found there based on host plant preferences and were thus deemed non-resident prey (Table 4.3, Fig. 4.2) (Blackman and Eastop 1994, Blackman 2000). Non-resident prey was only detected in *H. convergens* predators, and just one individual of this species consumed only non-resident prey (*R. maidis*) (Table 4.4). Of the remaining 10 *H. convergens* individuals that consumed non-resident prey, they also consumed either *S. avenae* or *H. axyridis* resident prey. Only *H. convergens* individuals consuming one (*S. avenae*) or no prey species did not consume non-resident prey. *C. maculata* and *H. axyridis* individuals only consumed probable resident prey (Fig. 4.1, Table 4.3).

The effect of diet breadth, measured as prey richness, on the propensity of a predator to consume prey varied by both coccinellid species and category of prey (Table 4.6). Consumption of non-resident or resident prey did not depend on the diet breadth of any coccinellid species (Table 4.6). Consumption of all herbivore prey did not vary by diet breadth of any coccinellid species, yet *C. maculata* individuals were significantly more likely to eat herbivore-*Brassica* prey with broader diets (Table 4.6). The effect of prey richness on consumption of intraguild prey varied significantly by coccinellid predator species (Table 4.6). Only *H. convergens* individuals significantly increased probability of consuming intraguild prey with increasing diet breadth (Table 4.6).

**Discussion**

This study successfully detected prey in the majority of coccinellid individuals tested. By employing both a taxonomically broad metabarcoding and a species-specific MCA validation step we detected both herbivore and intraguild prey. Further, we detected both consumption of prey species observed in collection plots and unobserved resident and non-resident prey that likely would have been overlooked without molecular analysis (Table 4.2). The hierarchical process of first verifying prey detected within a pooled predator DNA sample, then in a constituent individual from that pooled sample with MCA allowed us to efficiently determine the diet breadth of 59 coccinellid individuals. This represents a tangible improvement from previous molecular diet analyses of arthropod predators, which faced trade-off between detecting a limited number of a priori identified prey species, or detecting a broad array of prey in fewer predator individuals due to the cost of metagenomic sequencing (Chen et al. 2000, Paula et al. 2016, Yang et al. 2017). Results from this study capture the complexity of prey consumption among co-occurring coccinellids in even just one sample time point and should motivate the integration similar multi-molecular, hierarchical testing methods to improve understanding of arthropod predator trophic ecology.

In the sampled *Brassica* agroecosystem, the three coccinellid species tested consumed herbivore prey more often than intraguild prey. While all coccinellid species consumed aphid prey, only one species, *Coleomegilla maculata* consumed lepidopteran prey likely to cause damage to the focal *Brassica* crop and thus contributed pest management services (Weires and Chiang 1973, Hines and Hutchison 2001). Only one prey species, *Sitobion avenae* was shared amongst all coccinellid species, indicating low overlap in dietary niche, however intraguild prey consumption occurred with similar frequency among all coccinellid species. Diet breadth, measured as prey richness increased the likelihood of *C. maculata* consuming herbivore-*Brassica* pest prey, but also increased the likelihood of *H. convergens* consuming intraguild prey. The results also indicate that some *H. convergens* individuals may be recent immigrants to study plots, having fed upon non-resident prey within the last 5-48 hours likely captured by these molecular methods (Greenstone et al. 2010, Paula et al. 2016).

Coccinellids in the subfamily Coccinellinae are generally aphidophagous, however alternative prey can provide alternative or supplementary nutrition (Harwood and Obrycki 2005, Evans 2009). Yet, the degree to which coccinellid species tested in this experiment accept and thrive on different species of prey can vary greatly (Michaud 2005, Hodek and Evans 2012). Herbivore-*Brassica* pests present at the time collection (*Myzus persicae*, *P. rapae*, and *T. ni*), may not be preferred prey for the coccinellids tested based on the low frequency of consumption in this study. No coccinellids tested consumed *M. persicae* aphids, while all three species consumed *S. avenae* aphids, likely present on the *Digitaria* sp. crabgrass weeds present (Table 4.1) (Blackman 2000). *M. persicae* density in the plot could have been too low for coccinellid predators to locate them at the plot level (Table 4.2). Depending on whether coccinellid species spent more time foraging on *Brassica* leaves or weed plants, encounter rates with each aphid species would vary. *H. convergens* consumed an aphid which moves from a primary host (*Populus* sp.) to overwinter on *Brassica* roots in late summer and fall, *P. populitransversus,* suggesting a different foraging habitat domain than *C. maculata* which consumed foliar *Brassica* pests (Weires and Chiang 1973, Setzer 1985). Coccinellid species are known to partition space within a habitat, and this could have occurred in this *Brassica* agroecosystem based on differential prey use among coccinellid predators tested (Snyder 2009).

Intraguild prey was only detected in two *C. maculata* individuals, one of which likely consumed its intraguild prey, parasitoid *A. ervi,* by attacking a parasitized aphid prior to mummification (Meisner et al. 2011). Though these methods could not differentiate between prey life stages, instances of intraguild predation amongst coccinellid species were likely instances of adult coccinellids preying upon eggs or small larval stages of other species (Gardiner et al. 2011). Over half of *H. axyridis* and *H. convergens* individuals had consumed other coccinellid species, whereas only 5% of *C. maculata* individuals consumed coccinellid prey. Previous molecular gut content analysis found that half of *H. axyridis* and *C. maculata* individuals in soybean consumed other coccinellid species (Gagnon et al. 2011). It is unclear why *C. maculata* individuals consumed fewer coccinellid prey in this study, but it could be related to differences in habitat domain or ovipositional preferences among coccinellid species (Sicsú et al. 2015,

Greenop et al. 2018). *C. maculata* consumption of *Brassica* lepidopteran pests could indicate that this species spent more time foraging on *Brassica* vegetation than the other two species and encountered vulnerable younger coccinellid larvae and eggs less often. Alternatively, the lower consumption of coccinellid prey observed in *C. maculata* could be due to a preference for herbivore prey present in *Brassica* agroecosystems as compared to herbivores in soy or other agroecosystems. This could be explored further with controlled choice tests, including combinations of intraguild and extraguild prey taxa present in an given agroecosystem simultaneously.

Co-occurrence of predators and potential prey is an efficient way to understand which species may interact in an ecosystem, however these results highlight the limitations of surveys to accurately characterize trophic interactions. Had we relied solely upon field surveys to determine interactions, we would have overestimated the contribution of these coccinellids to herbivore population control, based on the assumption that coccinellids consume alternative prey opportunistically in field settings (Hodek and Evans 2012). This assumption may be necessary when access to predation verification methods such as video surveillance or molecular diet analysis is limited. However, researchers are increasingly able to merge field and molecular ecological studies to better interrogate relationships between species presence, interaction, and function (Brown et al. 2014, Furlong et al. 2014, Eitzinger et al. 2019).

The unexpected presence of non-resident prey in *H. convergens* individuals highlights the need to consider movement between resource patches to establish predator function. Detection of two non-resident aphid species in *H. convergens, H. setariae* and *R. maidis*, indicate recent *H. convergens* immigration from nearby corn fields to the *Brassica* plots (Table 4.3). Movement between habitat patches in an agricultural landscape is a common arthropod predator response to high rates of disturbances in ephemeral cropping habitats (Vasseur et al. 2013, Schellhorn et al. 2014). Determining predator diet breadths may serve as an indicator as to which predators are transient and which are actively providing predation services to control resident prey. Determining whether a predator is a resident of an agroecosystem or passing through is clearer for arthropod predator groups where only a less mobile larval stage consumes prey such as lacewings (Neuroptera) and hover flies (Syrphidae). As coccinellids continue to consume

prey as adults, departure from a given cropping patch may alter control of resident herbivore populations.

Diet breadth had varying effects on the propensity of coccinellids to consume differing types of prey. With a broader diet, *C. maculata* increased likelihood of consuming herbivore-*Brassica* pest prey, indicating a positive effect of prey diversity on predator function as a *Brassica* pest control provider. Conversely, when *H. convergens* broadened the number of prey taxa it consumed its likelihood of consuming intraguild prey increased significantly, indicating a negative effect of prey diversity on herbivore control in this agroecosystem. Providing alternative prey sources is often cited as a method to increase predator function in agroecosystems (Gurr et al. 2017), but rarely is consumption of alternative prey actually quantified (Harwood and Obrycki 2005). Until recently, molecular predator diet analysis has focused largely on prey species of economic importance due to relying upon species specific approaches (Furlong 2015). These results provide evidence to support previous claims that multiple tools are needed to understand coccinellid food webs (Weber and Lundgren 2009). Both broad metabarcoding and narrow species-specific molecular tools were required to detect and verify predator-prey relationships. However, detecting cannibalism or interactions among coccinellid life stages in future field studies would necessitate the incorporation of a third ELISA based method (Hagler et al. 2020).

Coccinellid prey consumption likely benefited and disrupted herbivore population regulation in model *Brassica* agroecosystems. In applied settings, identifying which predators complement rather than antagonize each other is increasingly recognized as an element of successful pest control (Crowder and Jabbour 2014). Just as predators which minimize overlap in foraging habitat may minimize antagonistic interactions by partitioning space, those which minimize overlap in shared prey could avoid antagonistic interactions by minimizing competition (Greenop et al. 2018). In this study, few prey taxa were shared among coccinellid predators, however, intraguild prey were commonly consumed (Rosenheim 1998). Both competition and intraguild predation contribute to interference among predator taxa, however the relative importance of each type of interaction to the ability of a predator assemblage to control herbivore prey remains unresolved and is likely agroecosystem and predator species dependent. Additional field

molecular diet analysis across ecosystems and seasons is needed to better understand why coccinellids vary in consumption of herbivore, intraguild, resident, and non-resident prey and how to enhance consumption of herbivore prey through purposeful management of plant and prey resources.

**Table 4.1** List of plant taxa observed in collection *Brassica* plots

| family | scientific name | common name | plot1 | plot2 |
|---|---|---|---|---|
| Amaranthaceae | *Amaranthus* | pigweed | | x |
| Amaranthaceae | *Chenopodium berlandieri* | lamb's quarter | | x |
| Brassicaceae | *Brassica oleracea* | collards | x | x |
| Fabaceae | *Vicia* | vetch | | x |
| Malvaceae | *Abutilon theophrasti* | velvet weed | | x |
| Poaceae | *Digitaria* | crabgrass | x | x |
| Portulaceae | *Portulaca oleracea* | purslane | x | x |
| Solanaceae | *Solanum* | horse nettle | | x |

**Table 4.2.** Per plant densities of arthropod taxa observed in *Brassica* plots by guild

| guild | family | species | plot 1 | plot 2 |
|---|---|---|---|---|
| herbivore | Aphididae | *Myzus persicae* | 2.42 | 1.42 |
| | Chrysomelidae | *Phyllotreta striolata* | - | 0.08 |
| | Pentatomidae | | - | 0.08 |
| | Pieridae | *Pieris rapae* | 2.41 | 2.08 |
| natural enemy | Anthocoridae | *Orius insidiosus* | - | 0.08 |
| | Araneae | | - | 0.08 |
| | Braconidae | *Aphidius* | - | 0.25 |
| | Coccinellidae | *eggs | 0.08 | 4 |
| | Chrysopidae | *eggs | 0.08 | 0.58 |
| | Pholcidae | | 0.17 | - |

**Table 4.3.** Prey consumed by coccinellid predators organized by guild and indicated if resident or non-resident based on host availability in collection plots (Table 4.1)

| guild | type | prey taxa | resident | non-resident | host |
|---|---|---|---|---|---|
| herbivore | lepidopteran | *Trichoplusia ni* | X | | *Brassica oleracea* |
| | | *Pieris rapae* | X | | *Brassica oleracea* |
| | aphid | *Sitobion avenae* | X | | *Digitaria sp.* |
| | | *Pemphigus populitransversus* | X | | *Populus sp. (1º) & Brassica sp. (2º)* |
| | | *Hysteroneura setariae* | | X | *Prunus sp. (1º)* or *Sorghum/Eleusine sp. (2º)* |
| | | *Rhopalosiphum maidis* | | X | *Zea mays* |
| natural enemy | parasitoid | *Aphidius ervi* | X | | *Sitobion avenae* & other *Aphididae* sp. |
| | coccinellid | *Harmonia axyridis* | X | | - |
| | | *Cycloneda munda* | X | | - |

**Table 4.4.** Number of individuals in a coccinellid species in which different types of prey were detected by count (n) and percentage of total individuals tested.

| **prey classification** | | | ***C. maculata*** | | ***H. axyridis*** | | ***H. convergens*** | | **all species** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **location** | **guild** | **type** | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** |
| resident | herbivore | all | 15 | 0.75 | 6 | 0.32 | 13 | 0.65 | 34 | 0.58 |
| | | *Brassica*-pest | 8 | 0.40 | - | - | - | - | 8 | 0.14 |
| | natural enemy | all | 2 | 0.10 | 4 | 0.21 | 8 | 0.40 | 14 | 0.24 |
| | | coccinellid | 1 | 0.05 | 4 | 0.21 | 8 | 0.40 | 13 | 0.22 |
| | all | | 16 | 0.80 | 7 | 0.37 | 14 | 0.80 | 37 | 0.63 |
| non-resident | herbivore | all | - | - | - | - | 11 | 0.55 | 11 | 0.19 |
| | | **total prey** | 16 | 0.80 | 7 | 0.37 | 15 | 0.75 | 38 | 0.64 |
| | | **total no prey** | 4 | 0.20 | 12 | 0.63 | 5 | 0.25 | 21 | 0.36 |

**Table 4.5.** Summary of generalized linear models testing whether consumption of a type of prey depends upon coccinellid species. Maximum likelihood estimates are given for models with significant species effects. Only one intraguild (natural enemy) or coccinellid prey species was consumed by an individual coccinellid predator, therefore these as well as the proportional responses were modeled as binomial responses. All other responses of prey types were modeled as Poisson responses.

| **response** | **d.f.** | **Wald $\chi^2$** | ***p*** | **predator species** | **MLE** | **SE** | **Wald $\chi^2$** | ***p*** |
|---|---|---|---|---|---|---|---|---|
| intraguild | 2 | 4.59 | 0.101 | | | | | |
| intraguild- coccinellid | 2 | 5.62 | *0.060* | *C. maculata* | -2.94 | 1.03 | 9.94 | **0.004** |
| | | | | *H. axyridis* | -1.32 | 0.56 | 5.52 | 0.019 |
| | | | | *H. convergens* | -0.41 | 0.46 | 0.31 | 0.374 |
| herbivore richness | 2 | 10.66 | **0.005** | *C. maculata* | -2.30 | 0.71 | 6.08 | **0.001** |
| | | | | *H. axyridis* | -1.56 | 0.50 | 7.67 | **0.002** |
| | | | | *H. convergens* | -0.92 | 0.35 | 1.60 | **0.010** |
| herbivore-*Brassica* pest richness | 2 | 0.00 | 1.000 | | | | | |
| resident richness | 2 | 6.14 | **0.046** | *C. maculata* | 0.26 | 0.20 | 0.08 | 0.181 |
| | | | | *H. axyridis* | -0.64 | 0.32 | 1.30 | **0.042** |
| | | | | *H. convergens* | 0.14 | 0.21 | 0.04 | 0.503 |
| non-resident richness | 2 | 0.00 | 1.000 | | | | | |
| total prey richness | 2 | 11.24 | 0.004 | *C. maculata* | 0.26 | 0.32 | 0.08 | **0.042** |
| | | | | *H. axyridis* | -0.64 | 0.20 | 1.30 | 0.181 |
| | | | | *H. convergens* | 0.56 | 0.17 | 0.60 | **0.001** |
| prop. intraguild | 2 | 2.12 | 0.347 | | | | | |
| prop. intraguild-coccinellid | 2 | 7.44 | **0.024** | *C. maculata* | -3.43 | 0.93 | 13.52 | **0.001** |
| | | | | *H. axyridis* | -0.59 | 0.51 | 1.09 | 0.256 |
| | | | | *H. convergens* | -1.53 | 0.44 | 4.47 | **0.001** |
| prop. herbivore | 2 | 2.12 | 0.347 | | | | | |
| prop. herbivore – *Brassica* pest | 2 | 0.00 | 1.000 | | | | | |
| prop. resident | 2 | 0.00 | 1.000 | | | | | |
| prop. non-resident | 2 | 0.00 | 1.000 | | | | | |

**Table 4.6.** Summary of logistic regressions testing whether the probability of consuming a given type of prey depends on dietary breadth measured as prey richness by coccinellid species. Maximum likelihood estimates for coefficients are given for models with significant effects.

| **response** | **component** | **df** | **Wald $\chi^2$** | ***p*** | **species** | **component** | **MLE** | **SE** | **Wald $\chi^2$** | ***p*** |
|---|---|---|---|---|---|---|---|---|---|---|
| intraguild | species | 2 | 7.53 | 0.023 | *C.maculata* | intercept | -2.57 | 1.14 | 5.09E+00 | 0.028 |
| | prey richness:species | 3 | 8.25 | **0.041** | | prey richness | 0.27 | 0.66 | 1.68E-08 | 0.682 |
| | | | | | *H. convergens* | intercept | -20.41 | 2094.24 | 9.49E-05 | 0.992 |
| | | | | | | prey richness | 19.31 | 2094.24 | 2.86E+02 | 0.993 |
| | | | | | *H. convergens* | intercept | -6.10 | 2.38 | 8.49E-06 | 0.013 |
| | | | | | | prey richness | 2.67 | 0.94 | 5.46E+00 | **0.006** |
| intraguild-coccinellid | species | 2 | 4.87 | 0.088 | | | | | | |
| | prey richness:species | 3 | 5.74 | 0.125 | | | | | | |
| herbivore | species | 2 | 1.28 | 0.527 | | | | | | |
| | prey richness:species | 3 | 0.00 | 1.000 | | | | | | |
| herbivore-*Brassica* pest | species | 2 | 0.00 | 1.000 | *C.maculata* | intercept | -5.63 | 2.30 | 5.97E+00 | 0.015 |
| | prey richness:species | 3 | 10.21 | **0.017** | | prey richness | 3.64 | 1.46 | 1.98E-07 | **0.013** |
| | | | | | *H. convergens* | intercept | -21.57 | 8187.00 | 6.94E-06 | 0.998 |
| | | | | | | prey richness | 0.00 | 8921.00 | 1.68E-23 | 1.000 |
| | | | | | *H. convergens* | intercept | -21.57 | 10590.00 | 6.94E-06 | 0.998 |
| | | | | | | prey richness | 0.00 | 4758.00 | 7.80E-26 | 1.000 |
| resident | species | 2 | 0.00 | 1.000 | | | | | | |
| | prey richness:species | 3 | 0.00 | 1.000 | | | | | | |
| non-resident | species | 2 | 0.00 | 1.000 | | | | | | |
| | prey richness:species | 3 | 0.00 | 1.000 | | | | | | |

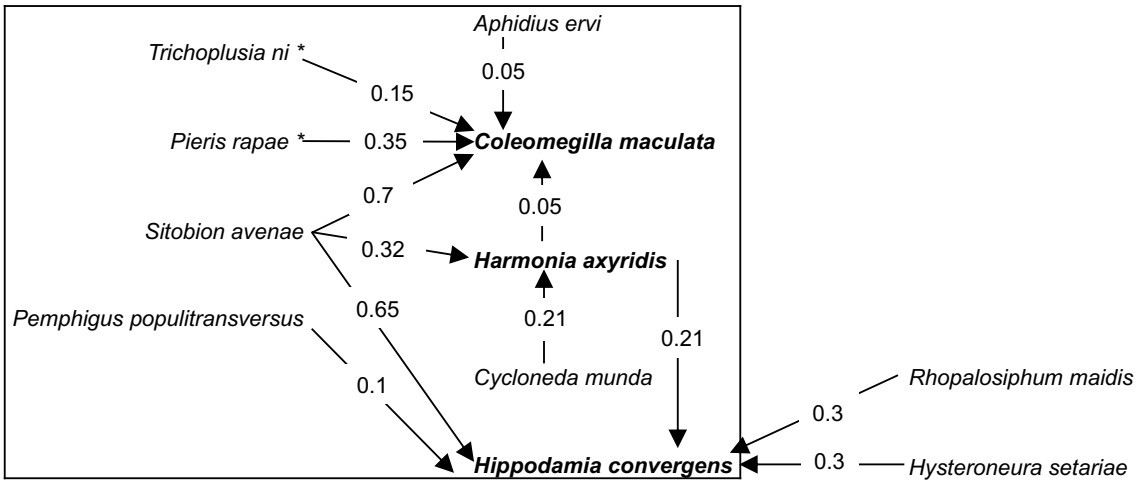



**Fig. 4.1.** Quantified predator-prey interaction network based on molecular gut-content analysis of focal coccinellid predators collected from two adjacent *Brassica* production plots combined. Arrows flow from prey to predator with proportion of predators testing positive for a given prey item indicated by number. Species in the left and right columns are herbivores with *Brassica* pests indicated with an asterisk. Species in middle column are natural enemies with focal coccinellid predators indicated in bold. Species within the box are resident taxa, whereas those outside the box are non-resident based on the absence of host plants from collection plots (Table 4.1).

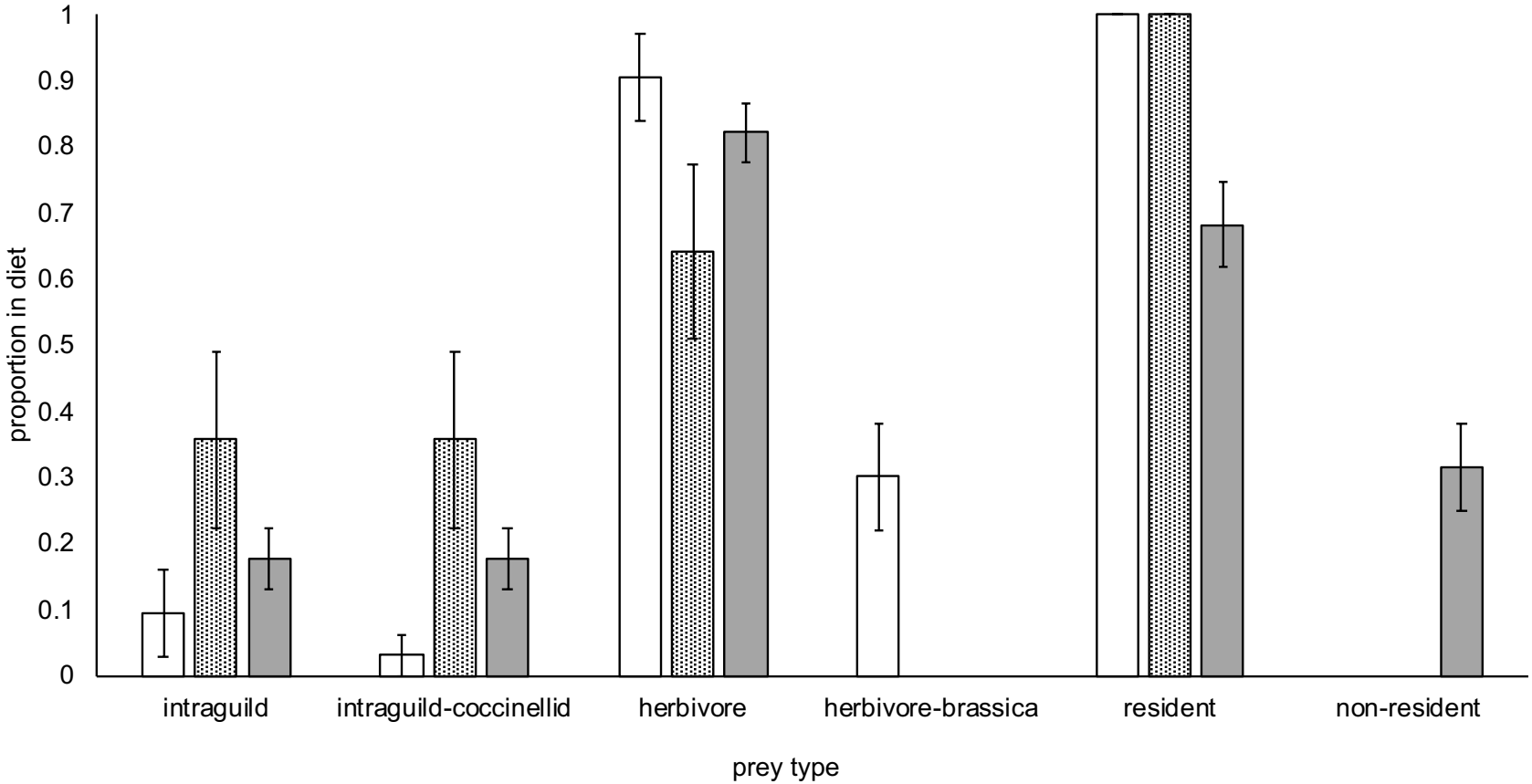



**Fig. 4.2.** Mean proportion of taxa by prey type in diets by coccinellid species. Bar fill pattern indicates coccinellid species: *Coleomegilla maculata* (white), *Harmonia axyridis* (dots), *Hippodamia convergens* (gray). Error bars indicate standard error.

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**Appendix 1**
**Chapter 1 Supplemental Information**

**Table S1.1** Summary results of generalized linear models of plant characteristics of study *Brassica* plots in the Federal District Brazil and Minnesota, United States at the 0.5m radius, plot, and farm scales. Statistical results are for Wald $\chi^2$ type 2 tests of main effects of country controlling for the nested factor of farm within country and year. Richness values modeled as Poisson responses; cover characteristics modeled as binomial responses.

| scale | predictor variable | factor | d.f. | Wald $\chi$2 | *p* |
|---|---|---|---|---|---|
| 0.5m radius | Plant richness * | climate | **1** | 0.40 | 0.526 |
| | | year | 1 | 0.23 | 0.631 |
| | | climate:farm | 7 | 2.70 | 0.911 |
| | Floral richness | climate | 1 | 1.01 | 0.315 |
| | | *year* | *1* | 5.61 | 0.018 |
| | | climate:farm | 7 | 5.00 | 0.660 |
| | Weed cover (%) | climate | 1 | 0.28 | 0.599 |
| | | year | 1 | 0.67 | 0.413 |
| | | climate:farm | 7 | 1.40 | 0.986 |
| | Floral cover (%) | climate | 1 | 0.33 | 0.568 |
| | | year | 1 | 0.13 | 0.716 |
| | | climate:farm | 7 | 0.52 | 0.999 |
| plot | Plant richness | **climate** | **1** | 18.37 | 0.000 |
| | | year | 1 | 0.14 | 0.705 |
| | | climate:farm | 7 | 8.06 | 0.328 |
| | Floral richness | **climate** | **1** | 3.46 | 0.063 |
| | | year | 1 | 1.24 | 0.265 |
| | | climate:farm | 7 | 5.86 | 0.557 |
| | Weed richness | climate | 1 | 9.02 | 0.003 |
| | | year | 1 | 0.05 | 0.824 |
| | | climate:farm | 7 | 7.82 | 0.349 |
| | Brassica cover (%) | **climate** | **1** | 0.87 | 0.351 |
| | | year | 1 | 0.15 | 0.696 |
| | | climate:farm | 7 | 0.22 | 1.000 |
| | Crop cover (%) | climate | 1 | 0.21 | 0.643 |
| | | *year* | *1* | 0.09 | 0.763 |
| | | climate:farm | 7 | 0.99 | 0.995 |
| | Weed cover (%) | climate | 1 | 0.23 | 0.628 |
| | | year | 1 | 0.00 | 0.997 |
| | | climate:farm | 7 | 1.72 | 0.974 |
| | Floral cover (%) | climate | 1 | 0.53 | 0.468 |
| | | year | 1 | 0.34 | 0.561 |
| | | *climate:farm* | *7* | 1.15 | 0.992 |
| | Alternative crop cover (%) | climate | 1 | 0.00 | 0.998 |
| | | year | 1 | 0.08 | 0.783 |
| | | climate:farm | 7 | 1.28 | 0.989 |
| | Bare (%) | climate | 1 | 0.06 | 0.808 |
| | | year | 1 | 0.11 | 0.744 |
| | | *climate:farm* | *7* | 0.54 | 0.999 |
| farm | Crop plant richness | climate | **1** | 76.77 | 0.000 |
| | | year | *1* | 9.34 | 0.002 |
| | | climate:farm | *7* | 43.43 | 0.000 |

**Table S1.2.** Summary results of linear models of arthropod characteristics of study *Brassica* plots in the Federal District Brazil and Minnesota, United States. Statistical results are for Wald $\chi^2$ type 2 tests of main effects of country controlling for the nested factor of farm within country and year. Responses were log transformed prior to model construction to meet assumptions of normality.

| response | predictor variables | d.f. | Wald $\chi^2$ | *p* |
|---|---|---|---|---|
| prey density | country | 1 | 4.99 | 0.026 |
| | year | 1 | 0.61 | 0.433 |
| | country:farm | 7 | 2.82 | 0.901 |
| prey richness | country | 1 | 5.28 | 0.022 |
| | year | 1 | 0.03 | 0.859 |
| | country:farm | 7 | 7.40 | 0.388 |
| predator to prey density | country | 1 | 3.09 | 0.079 |
| | year | 1 | 1.60 | 0.206 |
| | country:farm | 7 | 3.14 | 0.872 |
| predator to prey richness | country | 1 | 0.39 | 0.533 |
| | year | 1 | 1.02 | 0.312 |
| | country:farm | 7 | 3.98 | 0.782 |
| predator density | country | 1 | 0.00 | 0.965 |
| | year | 1 | 0.00 | 0.951 |
| | country:farm | 7 | 3.02 | 0.883 |
| predator richness | country | 1 | 2.44 | 0.119 |
| | year | 1 | 0.55 | 0.457 |
| | country:farm | 7 | 4.26 | 0.749 |

## Chapter 3 Supplemental Information

### Bioinformatic scripts

To check sequence quality:

```
$ fastqc <inputFileR1>
$ fastqc <inputFileR2>
```

To merge paired end reads based on a quality alignment score of 40 or higher for each lane of data:

```
$ illuminapairedend -score-min=40  <inputR1fwd> -r <inputR1rv>  >
<outputfileR1>
$ illuminapairedend -score-min=40  <inputR2fwd> -r <inputR2rv>  >
<outputfileR1>
```

To select only paired reads that passed quality filter:

```
$ obigrep -p 'mode!="joined"' <alignedinputR1> > <outputfilefilteredR1>
$ obigrep -p 'mode!="joined"' <alignedinputR2> > <outputfilefilteredR2>
```

To concatenate lanes into files:

```
$ cat <inputFileR1> <inputFileR2> > <outputR1R2>
```

To demultiplex file by primer-pair filters:

```
$ ngsfilter -t ngsfilter_table.txt -u <inputFile> > <assignedoutputFile>
```

To convert fastq to fasta format:

```
$ obiconvert --fasta-output <inputFastq> > <outputFasta>
```

To split fasta file into subfiles based on the attribute tag 'sample' which indicates primer-pair and biological sample:

```
$ obisplit -t sample > /directory
```

*Unclustered Bioinformatic Method 1: MEGAN*

To assign taxonomy:

```
$blastn -db <pathtodatabase> -query <inputFile> -evalue 1e-60 -max_target_seqs
25 -outfmt 5 -num_threads 12
```

Unclustered reads, split into samples by source predator and primer-pair uploaded to MEGAN (v.6.16.4) software and taxonomy parsed via default settings, except MinSupport ≥2 reads

*Clustered Bioinformatic Method 2-4: local-NCBI OTUblast, BOLD-NCBI loose & BOLD-NCBI strict*

To cluster reads we used the wrapper script NAPcluster (https://github.com/tjcreedy/NAPtime) to generate OTUs:

$NAPcluster --out <directory> --mode batch --threads 3 --verbose --seqlength <ampliconlength> --length_var 0.02 --minsize 2 --cluster_method usearch --cpv 3 --denoise no --usearch_version 92 <inputFile1> <inputFile2>…

*Bioinformatic Method 2: local-NCBI OTUblast*

To blast OTUs to a local database updated on 17/06/2018 comprised of only NCBI sequences:

$NAPcluster --out <directory> --mode batch --threads 3 --verbose --seqlength <ampliconlength> --length_var 0.02 --minsize 2 --cluster_method usearch --cpv 3 --denoise no --usearch_version 92 <inputFile1> <inputFile2>… --doblast --blastpath <pathtodatabase>

*Bioinformatic Method 3 & 4: BOLD-NCBI loose & BOLD-NCBI strict*

Used **BOLD_NCBI_MERGER** scripts from Macher et al. 2017 to create a database of all invertebrate sequences for both BOLD and NCBI

To assign taxonomy to BOLD-NCBI database with loose parameters:

$blastn -db <pathtodatabase> -query <inputFile> -evalue 1e-60 -max_target_seqs 25 -outfmt 5 -num_threads 12

*Loose methods:*
All hits considered.

*Strict methods:*
Only hits with >95% ID considered.

**CTAB protocol and DNA extraction method of the positive controls in the MCA**
Specimens were placed in 240 µl of CTAB buffer (100 mM Tris HCl pH 8.0; 20 mM EDTA pH 8.0; 1.4 M NaCl; 2% Cetyltrimethylammonium bromide (CTAB); 1% PVP-40; 0.2% (v/v) 2-mercaptoethanol; proteinase K added to 100 µg/ml) with two 4.5 mm zinc plated beads and homogenized for 60 s at 6.5 m/s in a FastPrep-24 Instrument. Beads were removed and samples were centrifuged at 10,000x*g* for 2 min at 25°C before incubation at 55°C for 2 h in a water bath. An equivalent volume of phenol/chloroform/isoamyl alcohol (25:24:1) as the sample was added and the sample tubes were centrifuged for 10 min under 18,705x*g* at room temperature. The upper aqueous phase was transferred to a clean microtube to which 3 M sodium acetate and isopropanol at approx. 1/10 of the sample volume was added to precipitate out the DNA at -20°C overnight. Pellets were recovered by centrifugation at 18,705x*g* for 20 min at 4ºC and the supernatant was discarded. Pellets were washed in 1 ml of 70% ice-cold ethanol twice, centrifuging at maximum speed at 4ºC for 15 min after each wash. Finally, pellets were dried at 60ºC for 5 min and resuspended in 50 µl dd$H_20$ overnight at 4ºC.

**Table S3.1.** Summary of the arthropod reference sequences downloaded with PrimerMiner (v0.15) for metabarcoding primer design at the family level. Sequences are available at the Data Repository for U of M (DRUM) (conservancy.umn.edu), found under the authors names.

| | | *cox1* seqs | | Mitogen seq | order summary | | |
|---|---|---|---|---|---|---|---|
| **Order** | **Family** | **BOLD** | **GenBank** | **GenBank** | **input seq** | **dereplicated** | **clustered (97% ID)** |
| Acari | Eriophyidae | 193 | 405 | 5 | 32213 | 15672 | 2587 |
| | Heterostigmata | 0 | 509 | 0 | | | |
| | Holothyrida | 1 | 1 | 1 | | | |
| | Mesostigmata | 16498 | 8338 | 12 | | | |
| | Phytoseiidae | 1609 | 791 | 7 | | | |
| | Tarsonemidae | 124 | 117 | 0 | | | |
| | Tenuipalpidae | 47 | 447 | 0 | | | |
| | Tetranychidae | 1303 | 1803 | 26 | | | |
| Araneae | Araneidae | 7779 | 5633 | 22 | 89819 | 41556 | 4345 |
| | Clubionidae | 2765 | 1616 | 0 | | | |
| | Linyphiidae | 16429 | 10696 | 0 | | | |
| | Lycosidae | 8842 | 5282 | 6 | | | |
| | Oxyopidae | 378 | 123 | 2 | | | |
| | Salticidae | 4169 | 2637 | 8 | | | |
| | Tetragnathidae | 3940 | 2689 | 5 | | | |
| | Theridiidae | 6642 | 4345 | 0 | | | |
| | Thomisidae | 3557 | 2272 | 3 | | | |
| Blattodea | | 3531 | 3155 | 707 | 7135 | 4456 | 1430 |
| Coleoptera | Anthicidae | 641 | 324 | 2 | 250109 | 122511 | 28441 |
| | Attelabidae | 288 | 209 | 4 | | | |
| | Bruchidae | 0 | 1031 | 12 | | | |
| | Buprestidae | 1050 | 908 | 12 | | | |
| | Cantharidae | 6752 | 4620 | 4 | | | |
| | Carabidae | 16573 | 14845 | 49 | | | |
| | Cerambycidae | 5825 | 5263 | 41 | | | |
| | Chrysomelidae | 14640 | 19108 | 234 | | | |
| | Cleridae | 1187 | 726 | 4 | | | |
| | Coccinellidae | 6381 | 5047 | 27 | | | |
| | Cryptophagidae | 1001 | 566 | 2 | | | |
| | Curculionidae | 22831 | 24774 | 171 | | | |
| | Dryophthoridae | 1337 | 1442 | 10 | | | |
| | Dynastidae | 0 | 0 | 0 | | | |
| | Elateridae | 6963 | 5203 | 24 | | | |
| | Elmidae | 1043 | 1025 | 3 | | | |
| | Erirhinidae | 0 | 309 | 1 | | | |

| | | *cox1* seqs | | Mitogen seq | order summary | | |
|---|---|---|---|---|---|---|---|
| **Order** | **Family** | **BOLD** | **GenBank** | **GenBank** | **input seq** | **dereplicated** | **clustered (97% ID)** |
| | Histeridae | 583 | 717 | 12 | | | |
| | Lagriidae | 0 | 3266 | 30 | | | |
| | Lucanidae | 838 | 1436 | 21 | | | |
| | Lycidae | 307 | 1229 | 4 | | | |
| | Melandryidae | 783 | 524 | 2 | | | |
| | Meloidae | 338 | 180 | 21 | | | |
| | Melyridae | 1139 | 559 | 7 | | | |
| | Mordellidae | 1730 | 709 | 6 | | | |
| | Mycetophagidae | 267 | 148 | 0 | | | |
| | Nitidulidae | 2154 | 1941 | 13 | | | |
| | Oedemeridae | 539 | 296 | 2 | | | |
| | Passalidae | 32 | 36 | 2 | | | |
| | Phalacridae | 868 | 415 | 0 | | | |
| | Ripiphoridae | 24 | 40 | 2 | | | |
| | Scarabaeidae | 5131 | 9058 | 100 | | | |
| | Scolytidae | 0 | 4810 | 82 | | | |
| | Silvanidae | 217 | 170 | 5 | | | |
| | Staphylinidae | 18664 | 12226 | 160 | | | |
| | Staphylinidae | 18664 | 12226 | 160 | | | |
| | Tenebrionidae | 2689 | 3266 | 30 | | | |
| | Tenebrionidae | 2689 | 3266 | 30 | | | |
| Collembola | | 98023 | 25749 | 24 | 123786 | 48557 | 5569 |
| Dermaptera | Forficulidae | 750 | 446 | 0 | 1229 | 390 | 42 |
| | Labiduridae | 21 | 11 | 1 | | | |
| Diptera | Agromyzidae | 12557 | 7369 | 14 | 1266620 | 507703 | 40571 |
| | Anisopodidae | 2679 | 1702 | 2 | | | |
| | Anthomyiidae | 38703 | 25047 | 3 | | | |
| | Asilidae | 2388 | 1143 | 2 | | | |
| | Bibionidae | 1691 | 1037 | 0 | | | |
| | Bombyliidae | 924 | 273 | 0 | | | |
| | Calliphoridae | 11434 | 9146 | 131 | | | |
| | Cecidomyiidae | 183547 | 103684 | 8 | | | |
| | Ceratopogonidae | 40172 | 20158 | 2 | | | |
| | Chloropidae | 16172 | 7542 | 0 | | | |
| | Conopidae | 222 | 168 | 0 | | | |
| | Cryptochaetidae | 0 | 0 | 0 | | | |
| | Culicidae | 31672 | 28137 | 143 | | | |
| | Dolichopodidae | 30545 | 15012 | 1 | | | |
| | Drosophilidae | 12781 | 12417 | 170 | | | |

| | | *cox1* seqs | | Mitogen seq | order summary | | |
|---|---|---|---|---|---|---|---|
| **Order** | **Family** | **BOLD** | **GenBank** | **GenBank** | **input seq** | **dereplicated** | **clustered (97% ID)** |
| | Empididae | 10362 | 6963 | 2 | | | |
| | Muscidae | 46507 | 27048 | 31 | | | |
| | Mycetophilidae | 37146 | 24274 | 0 | | | |
| | Pachyneuridae | 1 | 1 | 2 | | | |
| | Phoridae | 103847 | 56904 | 3 | | | |
| | Pipunculidae | 3435 | 1598 | 0 | | | |
| | Psilidae | 466 | 273 | 0 | | | |
| | Rhagionidae | 2218 | 1417 | 1 | | | |
| | Sarcophagidae | 7374 | 5306 | 27 | | | |
| | Sciaridae | 139992 | 72236 | 1 | | | |
| | Syrphidae | 16740 | 12052 | 9 | | | |
| | Tabanidae | 6387 | 4899 | 5 | | | |
| | Tachinidae | 26126 | 13686 | 8 | | | |
| | Tephritidae | 8704 | 12021 | 81 | | | |
| Hemiptera | Acanthosomatidae | 341 | 331 | 2 | 213478 | 89287 | 10556 |
| | Adelgidae | 1074 | 1292 | 1 | | | |
| | Aleyrodidae | 5113 | 9606 | 31 | | | |
| | Alydidae | 439 | 461 | 3 | | | |
| | Anthocoridae | 711 | 570 | 4 | | | |
| | Aphididae | 33060 | 22186 | 37 | | | |
| | Aphrophoridae | 1892 | 979 | 5 | | | |
| | Aradidae | 282 | 234 | 13 | | | |
| | Berytidae | 121 | 88 | 2 | | | |
| | Cercopidae | 133 | 416 | 16 | | | |
| | Cicadellidae | 49361 | 26926 | 40 | | | |
| | Coccidae | 966 | 1430 | 0 | | | |
| | Coreidae | 658 | 689 | 5 | | | |
| | Cryptococcidae | 3 | 305 | 0 | | | |
| | Delphacidae | 2385 | 1847 | 17 | | | |
| | Diaspididae | 777 | 855 | 0 | | | |
| | Eriococcidae | 275 | 305 | 0 | | | |
| | Eriosomatidae | 0 | 294 | 2 | | | |
| | Fulgoridae | 54 | 129 | 8 | | | |
| | Heterogastridae | 10 | 4 | 0 | | | |
| | Lygaeidae | 1585 | 1229 | 3 | | | |
| | Margarodidae | 24 | 1 | 0 | | | |
| | Membracidae | 1162 | 975 | 7 | | | |
| | Miridae | 12945 | 10050 | 24 | | | |
| | Nabidae | 858 | 577 | 10 | | | |

| | | *cox1* seqs | | Mitogen seq | order summary | | |
|---|---|---|---|---|---|---|---|
| **Order** | **Family** | **BOLD** | **GenBank** | **GenBank** | **input seq** | **dereplicated** | **clustered (97% ID)** |
| | Pentatomidae | 2032 | 2046 | 18 | | | |
| | Phylloxeridae | 276 | 316 | 1 | | | |
| | Phymatidae | 0 | 55 | 6 | | | |
| | Piesmatidae | 18 | 15 | 0 | | | |
| | Pseudococcidae | 2098 | 2159 | 0 | | | |
| | Psyllidae | 850 | 758 | 2 | | | |
| | Pyrrhocoridae | 82 | 84 | 2 | | | |
| | Reduviidae | 1137 | 1224 | 30 | | | |
| | Rhopalidae | 409 | 297 | 5 | | | |
| | Rhyparochromidae | 1311 | 880 | 2 | | | |
| | Tingidae | 690 | 542 | 7 | | | |
| Hymenoptera | Aphelinidae | 3304 | 1745 | 0 | 360699 | 179940 | 28156 |
| | Aphidiidae | 0 | 4415 | 1 | | | |
| | Argidae | 497 | 300 | 0 | | | |
| | Bethylidae | 1383 | 537 | 1 | | | |
| | Braconidae | 70031 | 49970 | 30 | | | |
| | Cephidae | 202 | 279 | 18 | | | |
| | Chalcididae | 305 | 137 | 0 | | | |
| | Cynipidae | 1522 | 1567 | 0 | | | |
| | Diprionidae | 455 | 340 | 0 | | | |
| | Encyrtidae | 2851 | 1641 | 0 | | | |
| | Eucoilidae | 0 | 973 | 1 | | | |
| | Eulophidae | 10726 | 5104 | 0 | | | |
| | Eurytomidae | 1793 | 1040 | 1 | | | |
| | Evaniidae | 169 | 147 | 2 | | | |
| | Figitidae | 4877 | 1885 | 1 | | | |
| | Ichneumonidae | 78252 | 43654 | 6 | | | |
| | Megaspilidae | 1410 | 2251 | 1 | | | |
| | Mymaridae | 13014 | 4679 | 0 | | | |
| | Pamphiliidae | 293 | 234 | 0 | | | |
| | Platygasteridae | 0 | 0 | 0 | | | |
| | Proctotrupidae | 577 | 284 | 0 | | | |
| | Pteromalidae | 6472 | 3732 | 11 | | | |
| | Scelionidae | 0 | 8349 | 3 | | | |
| | Siricidae | 269 | 357 | 0 | | | |
| | Tenthredinidae | 13016 | 8389 | 6 | | | |
| | Torymidae | 951 | 704 | 0 | | | |
| | Trichogrammatidae | 3847 | 1702 | 2 | | | |
| Isoptera | Kalotermitidae | 49 | 107 | 9 | 3226 | 1910 | 640 |

| | | *cox1* seqs | | Mitogen seq | order summary | | |
|---|---|---|---|---|---|---|---|
| **Order** | **Family** | **BOLD** | **GenBank** | **GenBank** | **input seq** | **dereplicated** | **clustered (97% ID)** |
| | Rhinotermitidae | 253 | 515 | 110 | | | |
| | Termitidae | 866 | 1012 | 542 | | | |
| Lepidoptera | Arctiidae | 0 | 14673 | 13 | 742823 | 307229 | 62427 |
| | Argyresthiidae | 1302 | 756 | 0 | | | |
| | Coleophoridae | 4939 | 2433 | 0 | | | |
| | Cossidae | 1423 | 837 | 3 | | | |
| | Crambidae | 37532 | 24705 | 61 | | | |
| | Gelechiidae | 25505 | 12401 | 10 | | | |
| | Geometridae | 83508 | 47464 | 32 | | | |
| | Gracillariidae | 14019 | 9107 | 3 | | | |
| | Hepialidae | 1693 | 1646 | 17 | | | |
| | Hesperiidae | 20929 | 18505 | 52 | | | |
| | Limacodidae | 3155 | 1868 | 6 | | | |
| | Lycaenidae | 12494 | 8760 | 19 | | | |
| | Lymantriidae | 0 | 4958 | 32 | | | |
| | Noctuidae | 75203 | 55877 | 60 | | | |
| | Nolidae | 5437 | 2106 | 4 | | | |
| | Notodontidae | 19744 | 15152 | 6 | | | |
| | Nymphalidae | 30333 | 24028 | 262 | | | |
| | Oecophoridae | 11458 | 6085 | 3 | | | |
| | Papilionidae | 3813 | 4613 | 83 | | | |
| | Pieridae | 7359 | 5049 | 45 | | | |
| | Pieridae | 7359 | 5049 | 45 | | | |
| | Plutellidae | 2323 | 1846 | 3 | | | |
| | Psychidae | 2476 | 1314 | 2 | | | |
| | Pterophoridae | 2410 | 974 | 1 | | | |
| | Pyralidae | 17165 | 7130 | 19 | | | |
| | Riodinidae | 2385 | 2330 | 4 | | | |
| | Sesiidae | 780 | 458 | 1 | | | |
| | Sphingidae | 13532 | 9390 | 7 | | | |
| | Tortricidae | 34224 | 19744 | 25 | | | |
| | Yponomeutidae | 1085 | 877 | 2 | | | |
| | Zygaenidae | 721 | 331 | 3 | | | |
| Mantodea | Mantidae | 534 | 453 | 18 | 998 | 586 | 236 |
| Neuroptera | Chrysopidae | 1277 | 963 | 15 | 4757 | 2627 | 264 |
| | Hemerobiidae | 1596 | 907 | 4 | | | |
| Orthoptera | Acrididae | 6817 | 6403 | 199 | 19634 | 11491 | 2275 |
| | Gryllidae | 972 | 948 | 21 | | | |
| | Tettigoniidae | 1916 | 2402 | 141 | | | |

| | | *cox1* seqs | | Mitogen seq | order summary | | |
|---|---|---|---|---|---|---|---|
| **Order** | **Family** | **BOLD** | **GenBank** | **GenBank** | **input seq** | **dereplicated** | **clustered (97% ID)** |
| Phasmatodea | Phasmatidae | 200 | 800 | 18 | 1010 | 618 | 146 |
| Psocoptera | | 0 | 8997 | 29 | 9012 | 5467 | 446 |
| Strepsiptera | | 254 | 367 | 6 | 625 | 385 | 128 |
| Thysanoptera | Aeolothripidae | 827 | 563 | 0 | 25347 | 9077 | 896 |
| | Phlaeothripidae | 1976 | 1535 | 2 | | | |
| | Thripidae | 11832 | 8607 | 12 | | | |

**Table S3.2.** Tags used in the primers for the metabarcoding analysis.

| Primer-sense | tag-name | sample description | Sequence (5'>3') |
|---|---|---|---|
| Forward | T1 | *Harmonia axyridis* – plot1 | TAATGA |
| | T2 | *Coleomegilla maculata* – plot1 | TCTTGG |
| | T3 | *Hippodamia convergens* – plot1 | CACTCT |
| | T4 | *Harmonia axyridis* – plot2 | CGTCAC |
| | T5 | *Coleomegilla maculata* – plot2 | TTCTCG |
| | T6 | *Hippodamia convergens* – plot2 | TGATCC |
| Reverse | R_tag | all samples – experiment tag | AGAGAC |

**Table S3.3.** qPCR program settings for the Melting Curve Analysis using SYBR Green I in LightCycler® 480 Instrument II to validate prey detection by metabarcoding.

| Program | Step | Temp (ºC) | Time | Ramp (ºC/s) | Cycles | Acquisition mode | Acquisition per ºC |
|---|---|---|---|---|---|---|---|
| 1. Pre-incubation | | 95 | 10 min | 4.4 | 1 | None | - |
| 2. Amplification | | | | | | | |
| Three-step | Denaturation | 95 | 15 s | 4.4 | 40 | None | - |
| or | Annealing | 53 | 30 s | 2.2 | 40 | None | - |
| | Extension | 72 | 30 s | 4.4 | 40 | Single | - |
| Two-step | Denaturation | 95 | 15 s | 4.4 | 40 | None | - |
| | Annealing/Extension | 60 | 60 s | 2.2 | 40 | Single | - |
| 3. Melting Curve | | | | | | | |
| | | 95 | 60 s | 4.4 | - | None | - |
| | | 40 | 60 s | 2.2 | - | None | - |
| | | 65 | 1 s | 1/1 | - | None | - |
| | | 95 | - | - | - | Continuous | 25 |
| 4. Cooling | | 40 | 10 s | 2.2 | 1 | None | - |

**Table S3.4.** Statistical results of hypothesis testing with ANOVA Type-II tests on generalized linear mixed models of metrics of metabarcoding efficacy performed with DNA sample as a random effect. All positives-type responses were modeled with a Poisson distribution and sensitivity and precision were modeled with a binomial distribution. Significant factors are displayed in bold text. RRA indicates relative read abundance.

| response | factor | $\chi^2$ | df | $p$ |
|---|---|---|---|---|
| positives | **primer** | **59.54** | **6** | **<0.001** |
| | method | 6.96 | 3 | 0.073 |
| | interaction | 7.14 | 18 | 0.989 |
| true positives | **primer** | **18.59** | **6** | **0.005** |
| | method | 2.61 | 3 | 0.455 |
| | interaction | 4.49 | 18 | 0.999 |
| sensitivity | **primer** | **12.64** | **6** | **0.049** |
| | method | 3.41 | 3 | 0.333 |
| | interaction | 4.63 | 18 | 0.999 |
| precision | length (bp) | 0.08 | 1 | 0.776 |
| | method | 0.28 | 3 | 0.964 |
| | interaction | 0.10 | 3 | 0.992 |
| false positives | **cluster** | **4.37** | **1** | **0.037** |
| | **primer** | **42.74** | **6** | **<0.001** |
| | interaction | 3.02 | 6 | 0.807 |
| true positives | RRA | 0.26 | 1 | 0.608 |
| | primer | 8.67 | 6 | 0.193 |
| | **interaction** | **31.30** | **6** | **<0.001** |
| RRA | **length (bp)** | **4.75** | **1** | **0.029** |
| | sample | 4.27 | 5 | 0.512 |
| | interaction | 0.35 | 5 | 0.997 |

**Table S3.5.** Number of reads assigned to a **false positive** prey taxon by each metabarcoding primer-pair, averaged across bioinformatic methods and coccinellid predators (*n*=6) detecting a given taxon. Numbers in parentheses indicate standard deviation.

| Order | Family | Species | 16S | Unimini | ArF10/R3 | BF1/R1 | BF1/agroR2 | agroF1/R1 | ZBJ |
|---|---|---|---|---|---|---|---|---|---|
| Collembola | Entomobryidae | *Entomobrya quadrilineata* | - | 15.00 (0) | - | - | - | - | - |
| Coleoptera | Coccinellidae | *Coleomegilla maculata* | 73956.00 (50867.85) | 33.00 (15.56) | 4490.75 (2574.26) | 128238.00 (73988.014) | 207774.00 (117598.75) | 335553.00 (191951.08) | 183072.00 (105666.62) |
| | | *Cycloneda munda* | - | - | - | - | - | 5.00 (0) | - |
| | | *Harmonia axyridis* | 98.50 (37.24) | 206849.00 (108180.61) | 3133.75 (1782.26) | 15950.00 (9020.52) | 21598.00 (12221.39) | 144337.25 (82361.40) | 50300.25 (33839.31) |
| | | *Hippodamia convergens* | 17.00 (0) | 175200.50 (98219.12) | 1562.75 (869.92) | 163579.25 (94257.19) | 14344.25 (8222.77) | 81577.00 (46067.80) | 99.25 (52.11) |
| Hemiptera | Aphididae | *Drepanaphis acerifoliae* | - | - | - | - | - | - | 6.00 (0) |
| | | *Hysteroneura setariae* | - | - | - | - | - | - | 6.00 (0) |
| | | *Macrosiphum euphorbiae* | - | - | - | - | - | - | 3.00 (0) |
| | | *Pemphigus populitranversus* | - | - | - | - | - | - | 8.00 (0) |
| | | *Sitobion avenae* | - | - | - | 1.00 (0) | - | - | - |
| | | *Orius insidiosus* | - | - | - | - | - | - | 66.00 (1.41) |
| Hymenoptera | Braconidae | *Aphidius ervi* | - | - | - | - | - | 13.00 | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | (0) | |
| | | *Dinocampus coccinellae* | - | 3.33 | 562.25 | 21.33 | 141.00 | 5779.75 | 526793.75 |
| | | | | (3.30) | (59.32) | (13.67) | (22.63) | (2537.23) | (492512.68) |
| Lepidoptera | Pieridae | *Pieris rapae* | - | 2.00 | - | 6.50 | 11.00 | 4.00 | 18314.33 |
| | | | | (0) | | (2.60) | (0) | (0) | (12940.02) |
| Thysanoptera | Thripidae | *Anaphothrips obscurus* | - | - | - | - | - | 465.00 | - |
| | | | | | | | | (0) | |
| | | All Taxa | 55578.25 | 382077.75 | 9749.50 | 307789.75 | 243831.00 | 567379.75 | 774063.25 |
| | | | (54487.96) | (206358.63) | (5167.04) | (177276.41) | (138046.20) | (322974.93) | (584289.29) |

**Table S3.6.** Number of reads assigned to a **false-positive** prey taxon for each bioinformatic pipeline, averaged across primer pairs and coccinellid predators (*n*=6) detecting the given taxon. Numbers in parentheses indicate standard deviation.

| Order | Family | Species | MEGAN | local-NCBI-OTUblast | BOLD-NCBI loose | BOLD-NCBI strict |
|---|---|---|---|---|---|---|
| Collembola | Entomobryidae | *Entomobrya quadrilineata* | 15.00 (0) | - | - | - |
| Coleoptera | Coccinellidae | *Coleomegilla maculata* | 1562.00 (1615.58) | 190686.67 (156232.06) | 179154.43 (147370.39) | 178700.71 (147140.81) |
| | | *Cycloneda munda* | - | 5.00 (0) | 5.00 (0) | 5.00 (0) |
| | | *Harmonia axyridis* | 3197.86 (6746.44) | 86003.57 (98680.21) | 86093.86 (98778.59) | 77428.57 (95992.04) |
| | | *Hippodamia convergens* | 1226.00 (1830.37) | 82818.00 (96690.27) | 82818.00 (96690.27) | 82670.71 (96639.14) |
| Hemiptera | Aphididae | *Drepanaphis acerifoliae* | - | 6.00 (0) | - | - |
| | | *Hysteroneura setariae* | - | - | 6.00 (0) | 6.00 (0) |
| | | *Macrosiphum euphorbiae* | - | 3.00 (0) | 3.00 (0) | 3.00 (0) |
| | | *Pemphigus populitranversus* | - | 8.00 (0) | 8.00 (0) | 8.00 (0) |
| | | *Sitobion avenae* | - | 1.00 (0) | 1.00 (0) | 1.00 (0) |
| | | *Orius insidiosus* | - | 65.00 (0) | 65.00 (0) | 65.00 (0) |
| Hymenoptera | Braconidae | *Aphidius ervi* | - | 13.00 | 13.00 | 13.00 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | (0) | (0) | (0) |
| | | *Dinocampus coccinellae* | 10565.67 | 5250.33 | 171227.00 | 171088.83 |
| | | | (22660.37) | (3358.86) | (379295.74) | (379008.14) |
| Lepidoptera | Pieridae | *Pieris rapae* | 9.00 | 6.25 | 5539.80 | 6819.00 |
| | | | (7.00) | (3.49) | (11067.10) | (11797.58) |
| Thysanoptera | Thripidae | *Anaphothrips obscurus* | - | 465.00 | - | - |
| | | | | (0) | | |
| | | All Taxa | 14648.57 | 334601.86 | 498804.14 | 489358.57 |
| | | | (20634.61) | (247231.12) | (423838.00) | (409426.63) |

**Table S3.7.** Model outcomes (log-odds) for true positive taxonomic assignment by metabarcoding dependent upon read abundance (RA) and primer. Sample was included as a random effect in the model. "16S" is the reference for categorical variable "primer". RRA: relative read abundance; SE: standard error calculated.

| variable | type | MLE | SE | Z-Value | *p* |
|---|---|---|---|---|---|
| Intercept | | -0.49 | 0.80 | -0.62 | 0.537 |
| RRA | | -1.84 | 1.23 | -1.49 | 0.136 |
| agroF1/R1 | primer | -0.42 | 0.83 | -0.51 | 0.610 |
| ArF10/R3 | primer | -1.03 | 0.89 | -1.16 | 0.246 |
| BF1/agroR2 | primer | 0.47 | 0.84 | 0.57 | 0.572 |
| BF1/R1 | primer | 0.76 | 0.85 | 0.90 | 0.369 |
| Unimini | primer | -0.26 | 0.88 | -0.30 | 0.763 |
| ZBJ | primer | -0.87 | 0.83 | -1.05 | 0.293 |
| RRA*agroF1/R1 | interaction | 1.41 | 1.42 | 1.00 | 0.319 |
| **RRA*ArF10/R3** | **interaction** | **2.90** | **1.42** | **2.05** | **0.041** |
| RRA*BF1/agroR2 | interaction | -1.89 | 1.76 | -1.08 | 0.282 |
| RRA*BF1/R1 | interaction | -2.33 | 1.79 | -1.30 | 0.194 |
| RRA*Unimini | interaction | 1.09 | 1.41 | 0.78 | 0.438 |
| **RRA*ZBJ** | **interaction** | **3.23** | **1.35** | **2.40** | **0.017** |

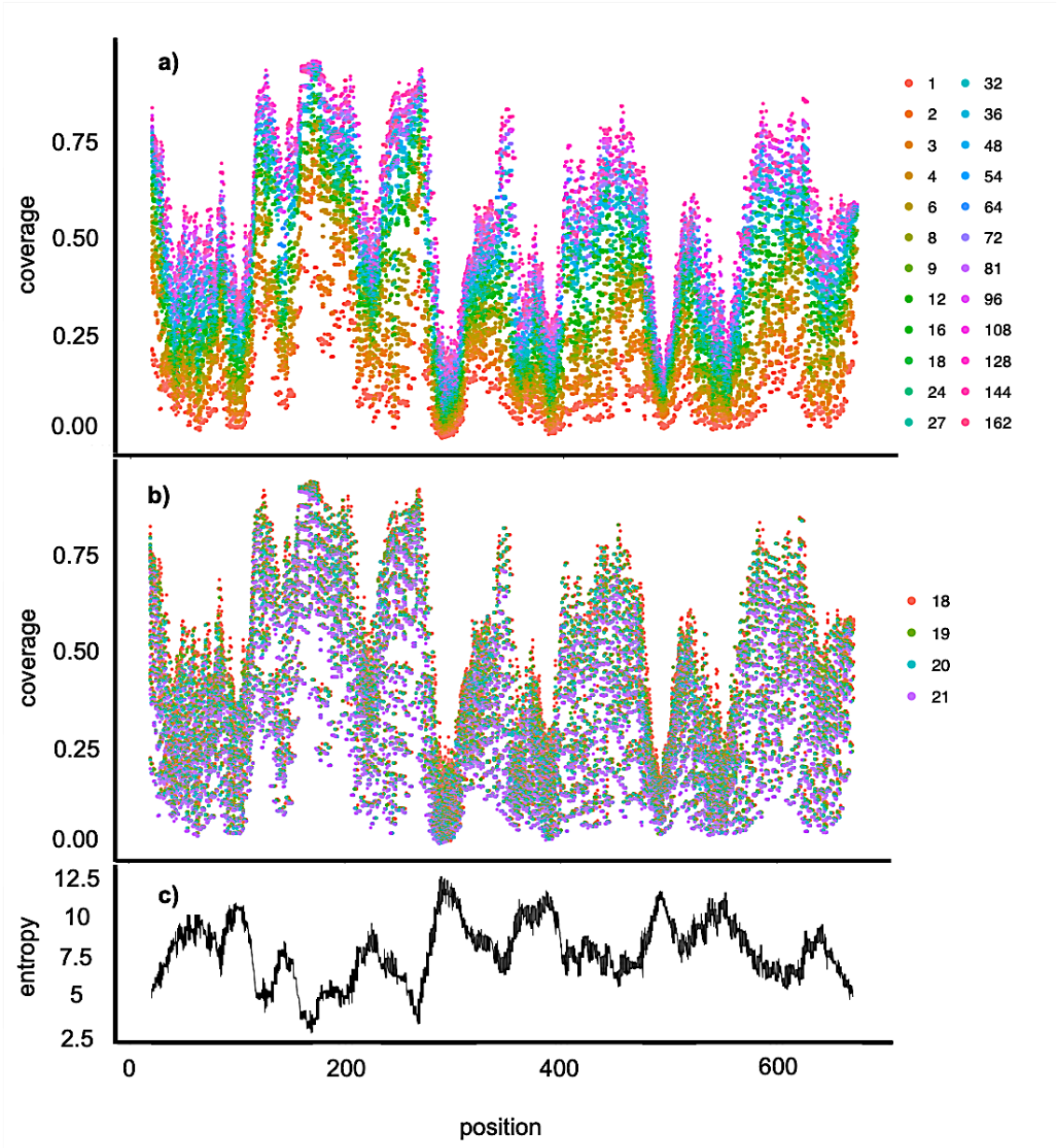


**Fig S3.1.** DegePrime generated primers for arthropods in agroecosystems along the Folmer region of the *cox1*gene mapping **a)** coverage by level of degeneracy, **b)** coverage by length (bp), and **c)** entropy along primer position.

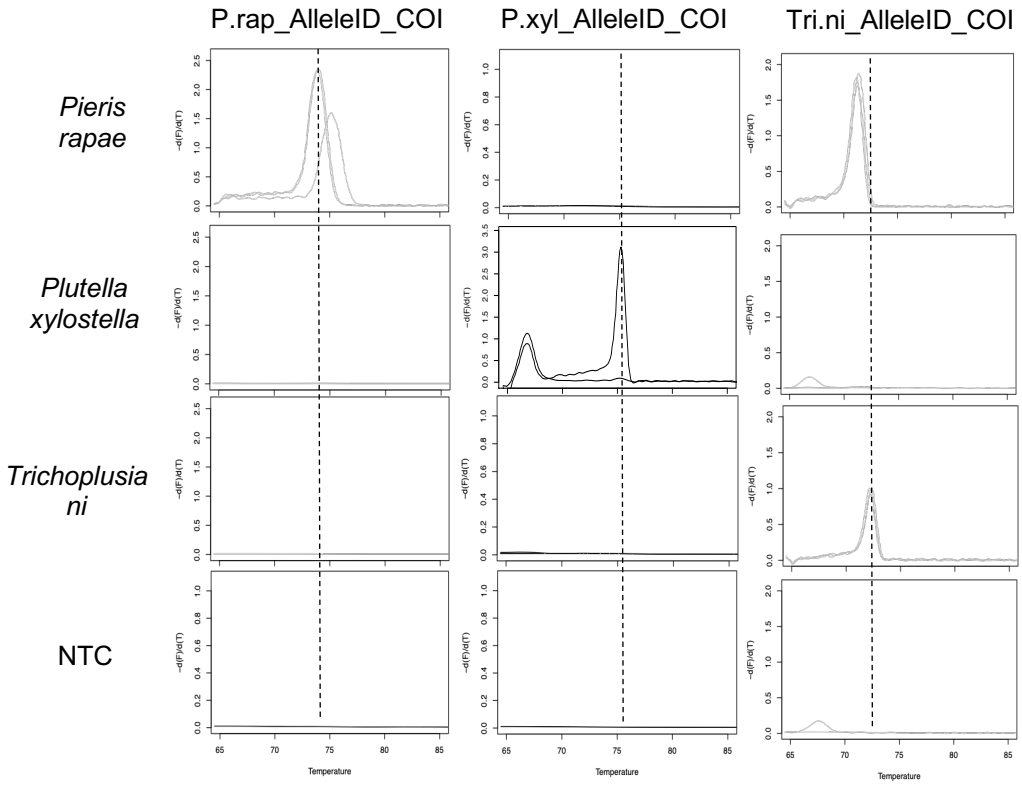


**Figure S3.2a**. Verification of qPCR primer specificity by melting curves analysis with qPCR SYBR- Green assays (x-axis = temperature, y-axis = -Δfluorescence/ Δtemperature). DNA source is indicated to the left of each row and primer is indicated above each column.

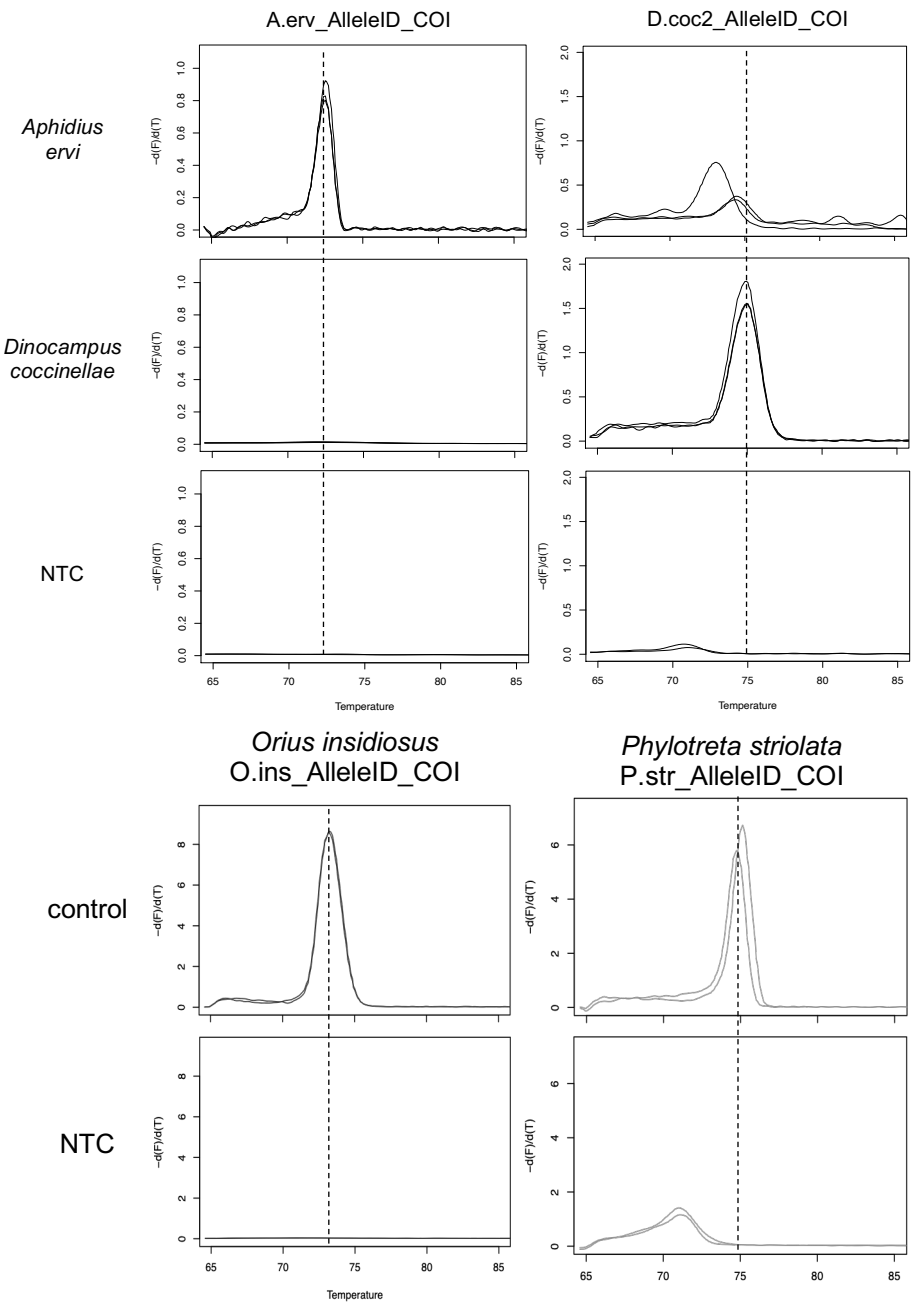


**Fig. S3.2b**. Verification of qPCR primer specificity by melting curves analysis with qPCR SYBR- Green assays (x-axis = temperature, y-axis = -Δfluorescence/ Δtemperature). DNA source is indicated to the left of each row and primer is indicated above each column.

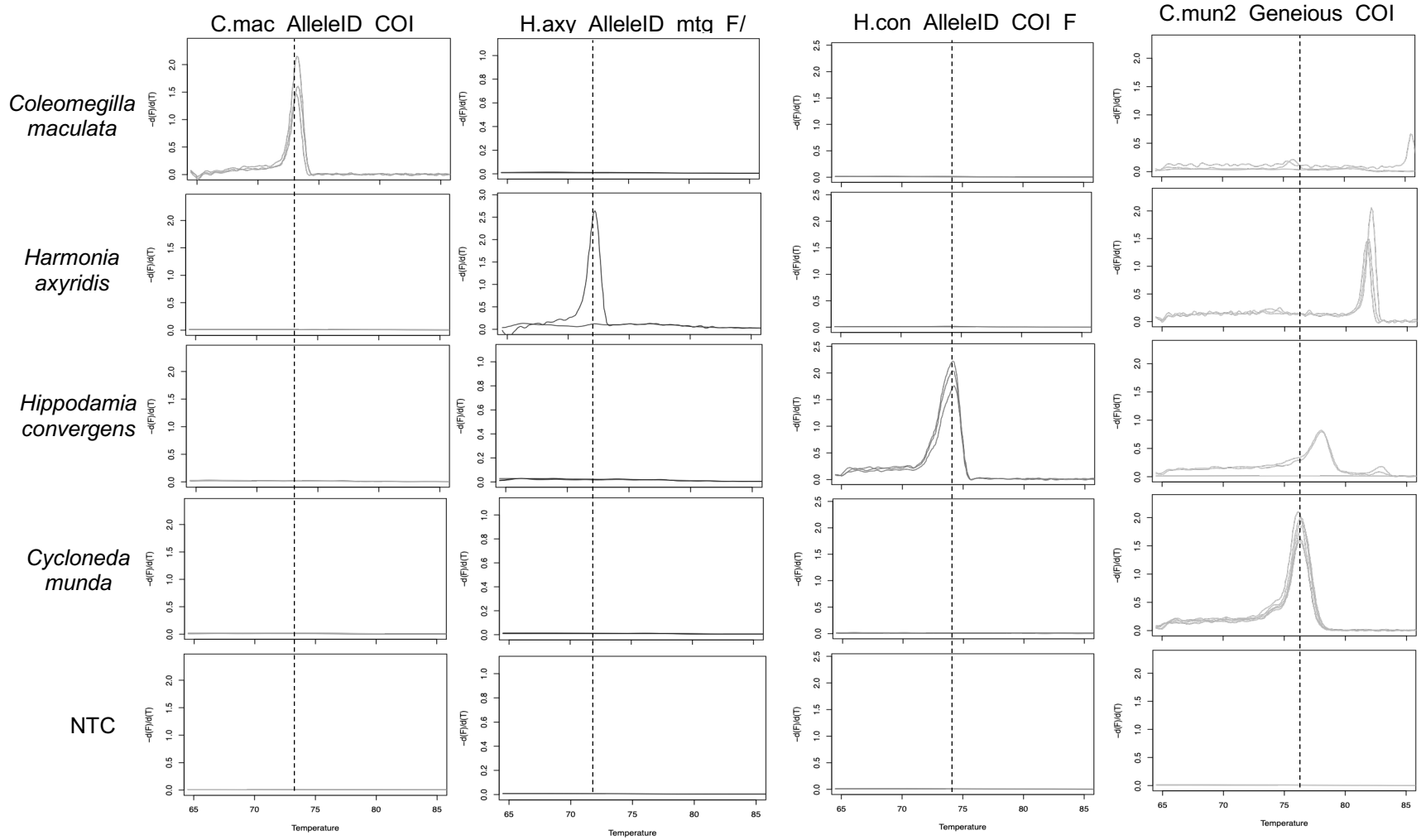


**Fig. S3.2c.** Verification of qPCR primer specificity by melting curves analysis with qPCR SYBR- Green assays (x-axis = temperature, y-axis = -Δfluorescence/ Δtemperature). DNA source is indicated to the left of each row and primer is indicated above each column.

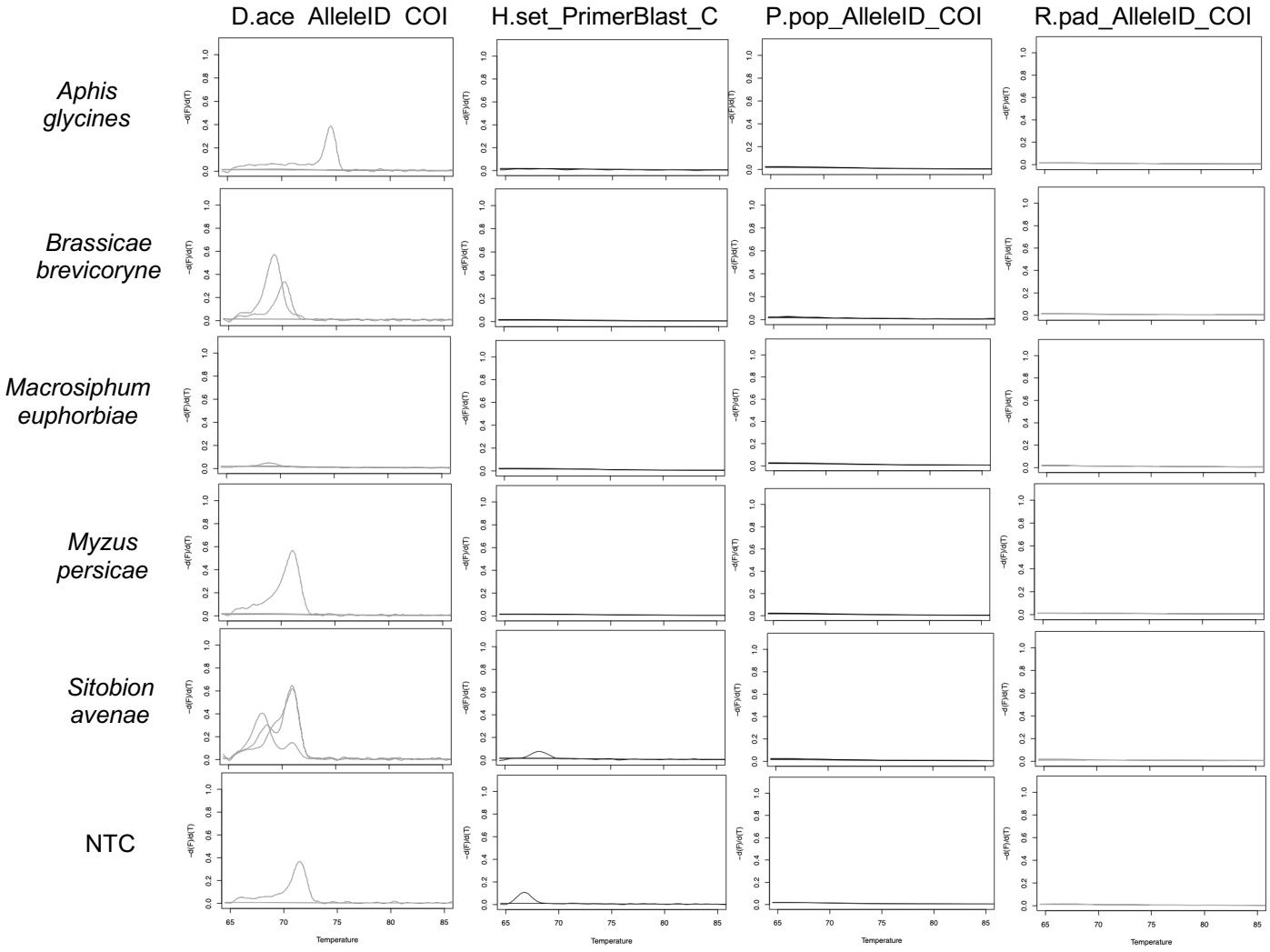


**Fig. S3.2d.** Verification of qPCR primer specificity by melting curves analysis with qPCR SYBR- Green assays (x-axis = temperature, y-axis = -Δfluorescence/ Δtemperature). DNA source is indicated to the left of each row and primer is indicated above each column.

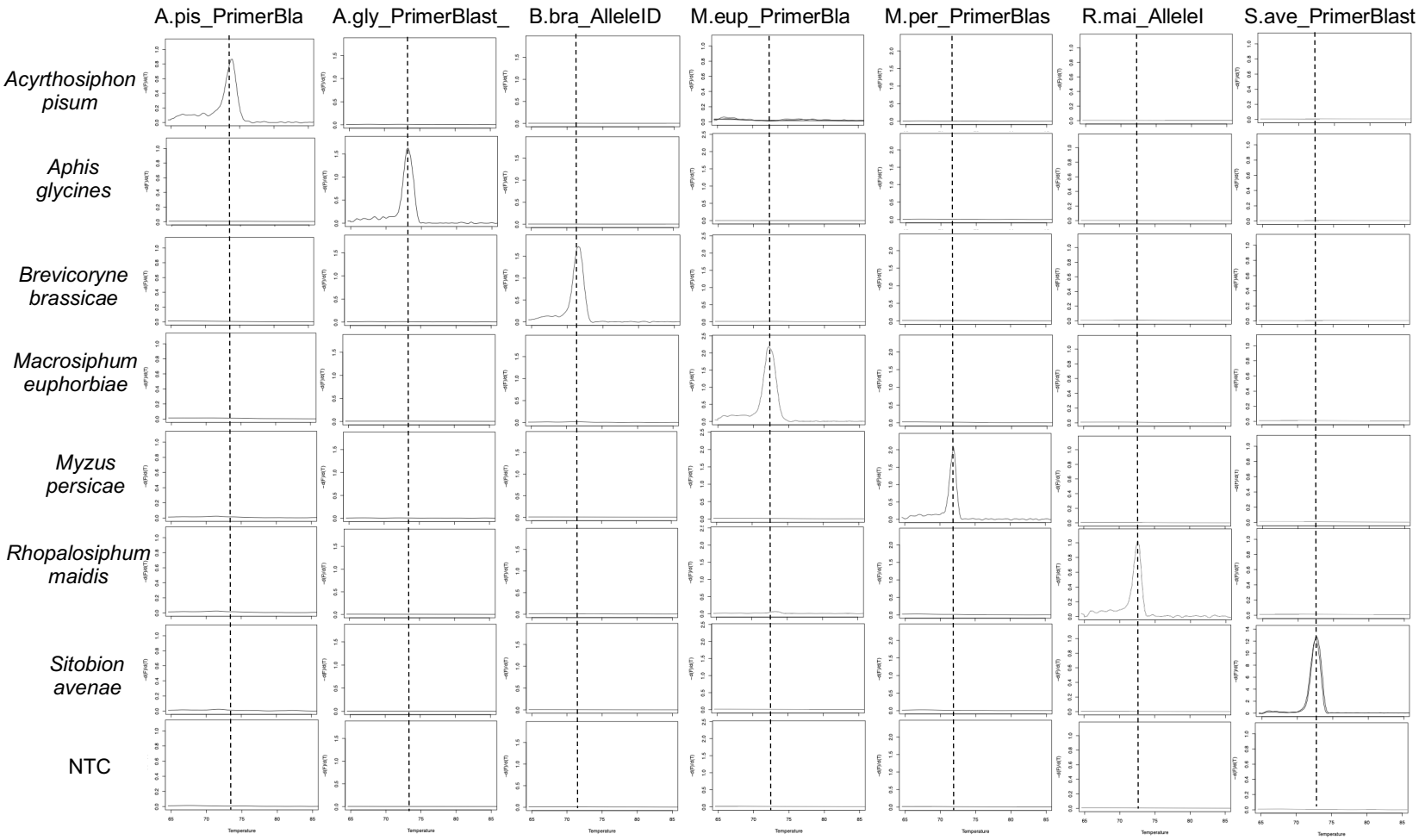


**Fig. S3.2e.** Verification of qPCR primer specificity by melting curves analysis with qPCR SYBR- Green assays (x-axis = temperature, y-axis = -Δfluorescence/ Δtemperature). DNA source is indicated to the left of each row and primer is indicated above each column.

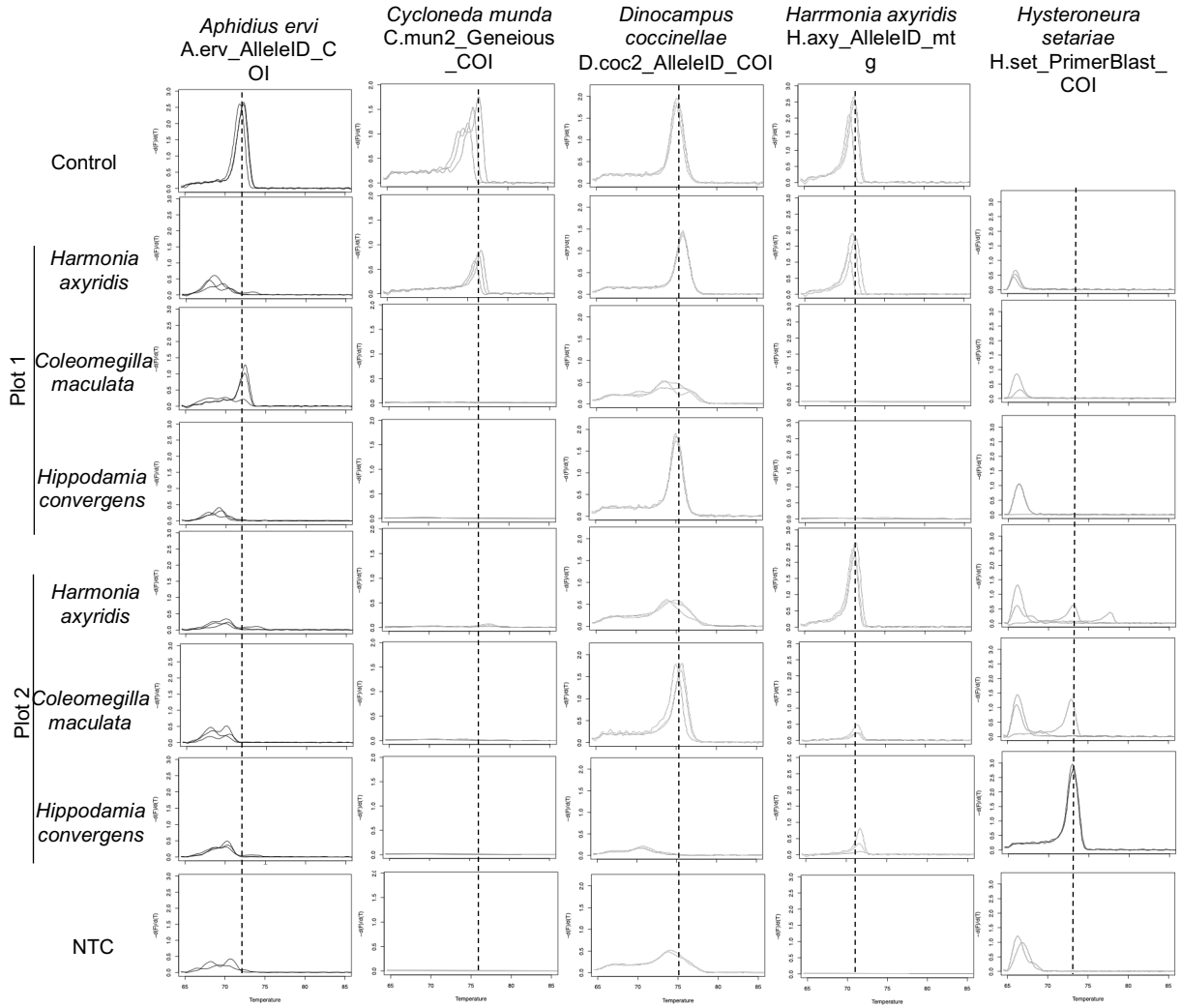


**Fig. S3.3a.** Detection of prey by melting curves analysis with qPCR SYBR- Green assays (x-axis = temperature, y-axis = -Δfluorescence/ Δtemperature). DNA source is indicated to the left of each row and primer is indicated above each column.

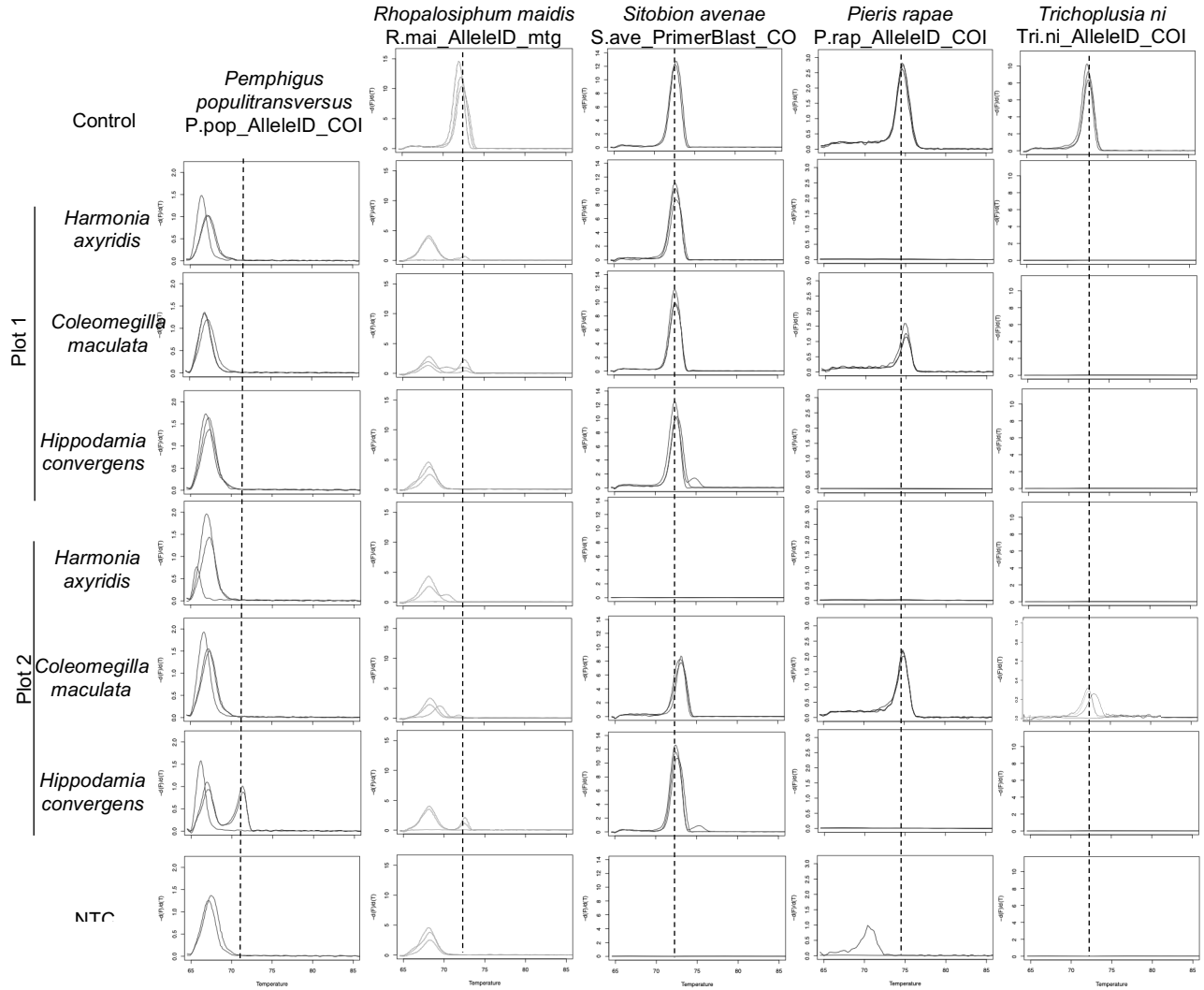


**Fig. S3.3b.** Detection of prey by melting curves analysis with qPCR SYBR- Green assays (x-axis = temperature, y-axis = -Δfluorescence/ Δtemperature). DNA source is indicated to the left of each row and primer is indicated above each column.

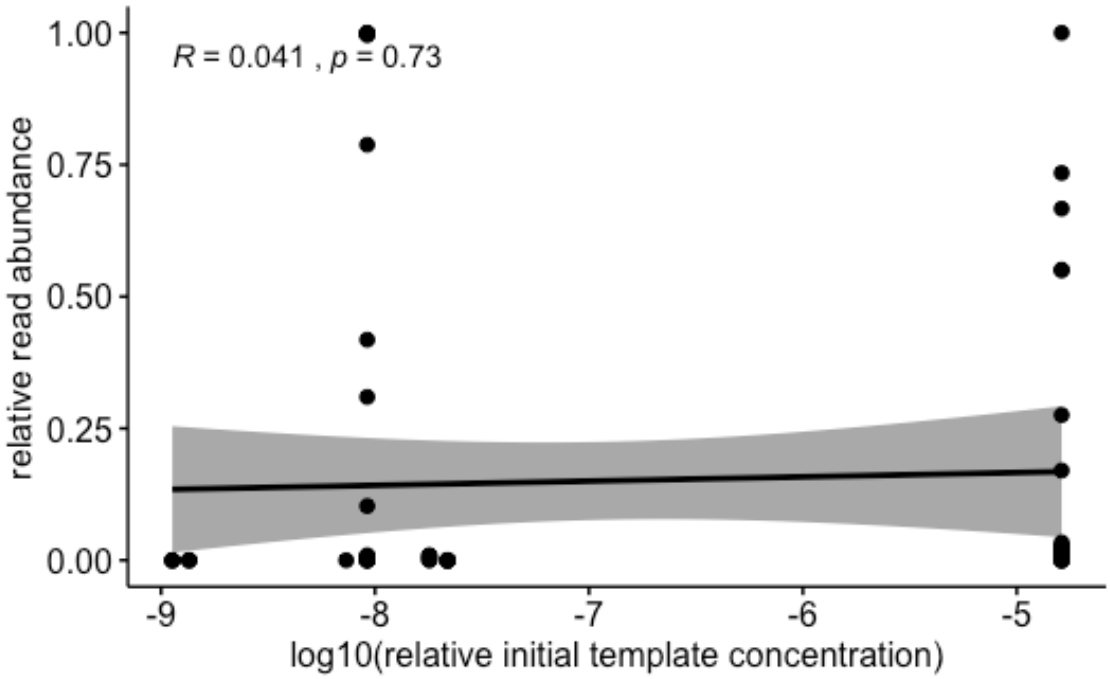


**Fig. S3.4.** Regression of relative initial prey template concentration estimated by melting curve analysis against metabarcoding relative read abundance (RRA) assigned to true positive prey in coccinellid gut contents.